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Calcium homeostasis and low-frequency magnetic and electric field exposure: A systematic review and meta-analysis of in vitro studies

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abstract

Low frequency magnetic field (LF MF) exposure is recurrently suggested to have the ability to induce health effects in society. Therefore, in vitro model systems are used to investigate biological effects of exposure. LF MF induced changes of the cellular calcium homeostasis are frequently hypothesised to be the possible target, but this hypothesis is both substantiated and rejected by numerous studies in literature. Despite the large amount of data, no systematic analysis of in vitro studies has been conducted to address the strength of evidence for an association between LF MF exposure and calcium homeostasis. Our systematic review, with inclusion of 42 studies, showed evidence for an association of LF MF with internal calcium concentrations and calcium oscillation patterns. The oscillation frequency increased, while the amplitude and the percentage of oscillating cells remained constant. The intracellular calcium concentration increased (SMD 0.351, 95% CI 0.126, 0.576). Subgroup analysis revealed heterogeneous effects associated with the exposure frequency, magnetic flux density and duration. Moreover, we found support for the presence of MF-sensitive cell types. Nevertheless, some of the included studies may introduce a great risk of bias as a result of uncontrolled or not reported exposure conditions, temperature ranges and ambient fields. In addition, mathematical calculations of the parasitic induced electric fields (IEFs) disclosed their association with increased intracellular calcium. Our results demonstrate that LF MF might influence the calcium homeostasis in cells in vitro, but the risk of bias and high heterogeneity (I^2 N 75%) weakens the analyses. Therefore any potential clinical implications await further investigation.

1. Introduction

Low-frequency magnetic fields (LF MF) generated by power distribution and usage have led to ever increasing public concerns regarding their potential to induce harmful biological effects. Some of the consequences commonly attributed, at least in part, to LF MF exposure range from non-specific physical symptoms such as sleep disorders and headaches (Schreier et al., 2006) to very specific diseases like childhood leukaemia (Ahlbom et al., 2000), breast cancer, and Alzheimer's disease (Davanipour and Sobel, 2009). However, direct evidence supporting an association between exposure and health status is thus far insufficient and inconsistent (Pedersen et al., 2014; Slusky et al., 2014; Liebl et al., 2015).

The concern for possible harmful health effects as well as scientific curiosity have led to the proposal of multiple potential mechanisms of action of LF MF on biological systems, as well as to a large pool of in vivo and in vitro experimental results (Prato, 2015; Barnes and Greenebaum, 2015). The common denominator of these studies is modulation of calcium homeostasis by LF MF. Cells from primary cultures or permanent cell lines (Simkó and Mattsson, 2004; McCreary et al., 2006; Carson et al., 1990; Conti et al., 1985a) are studied to explain or predict the mechanistic aspects of the observed interactions (Pilla et al., 2011; Pall, 2013; Gartzke and Lange, 2002). For example, it has been suggested that the cationic nature of the calcium ion might make it susceptible to the induced electric fields (IEF) generated by LF MF in solution (Gartzke and Lange, 2002; Lednev, 1991). The biological relevance of this presumed target for LF MF-cell interaction lies within the notion that calcium is an abundant and pivotal second messenger in the cell. Calcium signalling is crucial for cell function and survival (Missiaen et al., 2000; Khan et al., 1996) and functions as an intrinsic stressor to indicate cellular damage within minutes after the imposed insult (Steenbergen et al., 1987; Jeschke et al., 2009). Efficient calcium signalling requires maintenance of calcium homeostasis, with basal cytosolic calcium concentrations kept low and stable by storage of calcium ions in the endoplasmic reticulum (Berridge et al., 2003). However, upon activation, calcium is released from these stores into the cytoplasm and an intracellular signalling cascades is initiated. This subsequently regulates a

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second influx of extracellular calcium from the environment. Hence, calcium signalling results from a complex interplay between activation and inactivation of intracellular and extracellular calcium permeable channels. These fluxes of intracellular calcium can occur as transient increases or as repetitive calcium oscillations, which both ultimately lead to altered cell activity (Berridge et al., 2003; Smedler and Uhlen, 2014).

Over time, multiple potential targets of LF MF exposure and multiple mechanisms of interaction were proposed (Pilla et al., 2011; Pall, 2013; Gartzke and Lange, 2002), however conflicting results were obtained to either corroborating or refuting these theories. Some of the differences in experimental outcomes might be explained by the use of specific experimental parameters such as signal frequency (Niu et al., 2003), IEF (Liburdy, 1992), or specific cell type(s) sensitivity to exposures (Simkó and Mattsson, 2004; Pall, 2013). However, apparently conflicting in vitro results have obscured the support for any of these theories. On the other hand, further variability might be explained by unaccounted heterogeneity of the apparently controlled LF MF parameters of the exposure systems.

By now, sufficient data are present to systematically investigate the effect of LF MF exposure on calcium homeostasis. We conducted a systematic review and meta-analysis (Lau et al., 1997) for which we first selected a group of in vitro studies based on a strict set of quality criteria, on both the biological and the physical aspects of the reported data. Subsequently, we examined if the effects depend on the use of a particular cell type, type of calcium assay and specificities of magnetic or electric field exposure; frequency, magnitude, duration of exposure. Finally, we examined the reported biological effects in terms of possible differentials in LF MF exposure, introduced by unaccounted technical aspects of the exposure systems.

2. Methods

2.1. Study identification

The following electronic databases were searched to find any original articles concerning the effect of LF MFs on calcium in in vitro cell cultures (searched up until January 1, 2015): PubMed, Web of Science, Scopus, and EMBASE (via OvidSP). The search strategy was composed of three elements: LF MF, calcium and an element intended to exclude studies on high frequency fields, with a wide range of keywords and combinations adapted for every database/search engine (for full search strategy see Table 1). Furthermore, the reference lists of the selected papers and reviews were screened for potentially relevant papers that were not found with one of the four electronic databases. During the search, no language selection was applied. All inclusion criteria and methods of analysis were specified a priori in a protocol.

Table 1
Search strategy.

PubMed	
Calcium	Calcium [MeSH] OR calcium [tiab] OR CA [tiab] OR Ca^{2+} [tiab]
Exposure	(Magnetic or Electromagnetic Fields [mesh] OR (electromagnetic [tiab] AND field [tiab]) OR (electromagnetic [tiab] AND fields [tiab]) OR (electromagnetic [tiab] AND radiation [tiab]) OR (electromagnetic [tiab] AND radiations [tiab]) OR (electromagnetic [tiab] AND irradiation [tiab]) OR (electromagnetic [tiab] AND irradiations [tiab]) OR EMF [tiab] OR EMFs [tiab])
Exclusion	(radio[tiab] OR RF-EMF [tiab] OR RF-EMFs [tiab] OR static [tiab] OR MHz [tiab] OR megahertz [tiab] OR THz [tiab] OR terahertz [tiab])
Web of Science	
Calcium	TS = (calcium OR Ca OR Ca^{2+})
Exposure	TS = ((electromagn* near/3 (field* OR *radiation*)) OR EMF OR EMFs)
Exclusion	TS = (radio OR RF-EMF OR RF-EMFs OR static OR MHz OR megahertz OR THz OR terahertz)
Scopus	
Calcium	TITLE-ABS-KEY("calcium") OR TITLE-ABS-KEY ("ca") OR TITLE-ABS-KEY("Ca^{2+}")
Exposure	TITLE-ABS-KEY("Electromagnetic Field") OR TITLE-ABS-KEY("Electromagnetic")
Exclusion	TITLE-ABS-KEY("radio") OR TITLE-ABS-KEY("RF-EMF") OR TITLE-ABS-KEY ("MHz") OR TITLE-ABS-KEY("terahertz") OR TITLE-ABS-KEY("static")
EMBASE	
Calcium	Exp Calcium/OR (calcium OR Ca OR Ca^{2+}).tiab.
Exposure	(Exp electromagnetic field/OR Exp electromagnetic radiation/OR ((electromagnetic AND (field OR fields OR radiation OR radiations OR irradiation OR irradiations)) OR EMF OR EMFs).tiab.)
Exclusion	(radio OR RF-EMF OR RF-EMFs OR static OR MHz OR megahertz OR THz OR terahertz).tiab.

2.2. Study selection

The first screening of potentially relevant studies was performed based on title and abstract independently by two investigators (L.A. Golbach and B.M.L. van Kemenade). After screening, full text versions from the remaining papers were obtained if possible. All full text papers were evaluated based on the defined selection criteria by the same two investigators. Possible disagreement between investigators or technical uncertainties in the publications was resolved by a third investigator (L.A. Portelli). He also calculated technical uncertainties in the publications required to estimate the induced electrical fields.

For paper inclusion based on title and abstract, the following criteria were used:

Exposure: Only studies applying magnetic fields with frequencies between 1 and 300 Hz, no static magnetic fields.

Set-up: The studies should examine the effect of LF MF exposure on animal or human cells in an in vitro set-up. Studies that report direct animal exposure with subsequent analysis of individual cells were not included in the analysis, though isolation of cells from a primary source before exposure was included (ex vivo set-up). In addition, studies reporting experiments conducted on prokaryotes, algae, or fungi were excluded as well.

Reporting: The studies should report primary peer-reviewed data, reviews and meeting abstracts were not included.

For full-text inclusion, the following criteria were used:

Exposure: Only studies applying LF MFs with frequencies between 1 and 300 Hz were selected, no static magnetic fields. The exposure systems details needed to be reported in such a way that uncertainty regarding the exposure parameters could be reasonably quantised therefore allowing for the reproduction of the conditions. A complete description of the assumptions, estimations and calculations performed is found in supplementary note 1 and supplementary Table 3.

Calcium: The calcium assays reported in the studies should measure actual calcium release, uptake, fluctuations or homeostasis without the use of pharmacological inhibitors. Path-clamp experiments were excluded, since these measurements require short electrical pulses to depolarise the membrane and evoke calcium influx. Studies reporting deposition of solid calcium minerals were also excluded.

Set-up: The studies should examine the effect of LF MF exposure on animal or human cells in an in vitro set-up. Studies that report direct animal exposure with subsequent analysis of individual cells were not included in the analysis, though isolation of cells from a primary source before exposure was included (ex vivo set-up). In addition, studies reporting experiments conducted on prokaryotes, algae, or fungi were excluded as well.

Reporting: A language restriction was applied, only articles reporting in English were included in the analysis. The studies should report primary peer-reviewed data, so reviews and meeting abstracts were not included, however reviews were used to screen for missing articles. If the full text was not available online, neither through the library nor after contacting the authors, the article was excluded from the analyses.

2.3. Study characteristics and data extraction

For each included study, the following data were extracted: official cell type or cell line name, origin of cellular material, type of cells, dependent or independent control/sham groups, exposure frequency, duration and timing of exposure, magnetic flux density, type of calcium assay, batch or single measurements and stimulation of a calcium influx. Bibliographic details of the studies such as corresponding author, journal and year of publication were also retrieved (Table 1 Supplementary data).

From all studies (s,), number of events or mean, standard error (SE) or standard deviation (SD) and number of measurements or individual cells (n, individual experiments were denoted by capital letters) of control/sham and exposure groups were recorded. When the data from individual experiments or animals rather than aggregated data were presented (Conti et al., 1985a; Galvanovskis et al., 1996; Walleczek and Liburdy, 1990), the mean and SD were calculated. If data were only available in a graphical representation, values were measured with a digital ruler (Universal Desktop Ruler). Authors were contacted to obtain missing data on sample size, SD or SE. If a value was missing and authors did not respond, an estimate of the sample size was made by mathematical calculations with the SD or SE obtained from the graph(s) and the possible sample sizes reported in the paper. This was only performed for the data extracted from Lisi et al. (2006) and Pilger et al. (2004). For further specific details regarding these calculations, we contacted the corresponding author. Two experiments (Sakurai et al., 2005; Liu et al., 2014) and nine papers were excluded from the meta-analysis, as we were unable to obtain the required data (Tonini et al., 2001; Garciasancho et al., 1994; Lee et al., 2002; Hwang et al., 2011; Lindstrom et al., 1995; Nishimura et al., 1999; Grande et al., 1991; Oh et al., 2001; Conti et al., 1985b).

2.4. Assessment of risk of bias and reporting quality of included studies

The methodological quality of the included studies was determined using predefined criteria (Supplementary Table 2). For in vitro studies, no standard quality assessment tool exists; we therefore developed these criteria ourselves. Two reviewers (L.A. Golbach, B.M.L. van Kemenade) independently scored the selected papers for these criteria. The criteria shown in Table 2 were meant to assess the risk of systematic errors due to selection, performance or detection bias. The risk of these different biases was scored with "Low", "Moderate", or "High". When a paper lacked the necessary details to assess the risk, the risk was categorised as "Risk Unknown". Furthermore, we assessed the lack of reproducibility due to poor or incomplete reports (reporting quality). "No", "Partly" or "Yes", indicated the presence ("Yes/Partly") of absence ("No") of essential information regarding the study design and experimental controls.

2.5. Data synthesis and statistical analysis

First, the type of calcium assay used in each of the included reports was determined. From experiments that measured intracellular calcium concentrations with radioactive calcium (^{45}Ca) or with a fluorescence dye, the mean, SD/SE, and sample size (n) were extracted, to calculate standardised mean differences (SMD). The same was done for studies that described continuous data related to oscillations in calcium concentration. For studies in which only a number of events were described, an odds ratio was calculated. When outcomes were measured in independent experiments with different frequencies, magnetic flux density, or cell types, then all outcome values were noted. If the outcome of one sample was determined on multiple time points, being a dependent measurement in time, the moment with the largest difference between sham/control and LF MF treatment was selected for both the baseline and the stimulation moment. However, if outcomes were determined at different time points using separate independent samples, all time points were included. When the intracellular calcium concentration of resting cells, and subsequently the concentration during stimulation of a calcium influx, was measured under sham and LF MF conditions, both outcomes were noted (McCreary et al., 2006; Luo et al., 2014). From these double datasets, only data from the stimulated sample were used for the overall analysis.

The SMD, odds ratios, and effect sizes were calculated with specialised software, Comprehensive Meta Analysis (CMA version 2.0). Individual effect sizes were pooled to obtain an overall effect size and 95% confidence interval with a random effect size model. Based on the study characteristics of every experiment, subgroups were determined. Subgroup analyses were planned for the following study characteristics: the type of cells used, exposure frequency, magnetic flux density, duration of the LF MF exposure, and single cell or batch analysis of the intracellular calcium concentration. For the calcium concentration oscillation studies, individual analyses were planned for three types of outcome measures. All subgroups should consist of data from at least three individual papers or five independent experiments. The effect sizes and confidence intervals of the overall analyses and subgroup analyses were

Table 2
Reporting quality and risk of bias scheme.

		Yes	Partly	No	Risk unknown
Reporting quality	Is the cell origin and cell type used reported?	Reported	Not clearly reported	Not reported	–
	Is the duration of exposure reported?	Reported	Not clearly reported	Not reported	–
	Is the frequency of exposure reported?	Reported	Not clearly reported	Not reported	–
	Is the magnetic flux density of exposure reported?	Reported	Not clearly reported	Not reported	–
	Environmental background magnetic field reported	AC/DC reported	AC or DC reported	Not reported	
Performance bias	Is a sham or dummy coil used for control treatment?	Yes	–	No	Not reported
	Is the temperature controlled?	Yes, with SE	Yes, without SE	No	Not reported
	Was the exposure blinded?	Yes	–	No	Not reported
	Was the exposure randomised?	Yes	–	No	Not reported
Selection bias	Is the cell vitality scored/measured?	Yes	–	No	Not reported
Detection bias	Were the methods the same for control and exposure treatment?	Yes, independent measurements	Dependent measurements	No	–
	Were the data measurements randomised?	Yes	–	No	Not reported
Other bias	Was there no industry sponsoring involved	Yes	–	No	–

displayed in a forest plot. Heterogeneity was calculated in CMA and expressed as I^2, which is the proportion of variability in a meta-analysis that is explained by between-trial heterogeneity rather than by sampling error (Higgins and Thompson, 2002). We performed a sensitivity analysis to assess the robustness of our findings by removing studies in which we made calculated estimates of the mean, SD, or n (Baker and Jackson, 2008). If the direction of the effect depended heavily on the studies removed, then the results should be interpreted with great caution.

2.6. Electric and magnetic field exposure assessment

A thorough survey of the exposure conditions reported in literature was performed to extract all relevant information regarding the electric and magnetic field exposure parameters in the culture space. Only publications that provided enough explicit or implicit information about their exposure conditions were included in this review. Crosschecks were made between reported and calculated exposure values to establish the most accurate parameters of the magnetic and electric field exposure for each report. A complete description of the assumptions, estimations, and calculations performed is found in Supplementary Note 1 and Table S3. Briefly, the signal type, magnitude, spatial distribution, and geometric characteristics of the culture space were extracted for each exposure condition. Based on this data, maximum and minimum electric and magnetic fields to which cultures were exposed were calculated by means of the most extreme combination of exposure parameters to set the upper boundaries and uncertainties for each exposure condition. In the case of exposures under a microscope, calculations were also made based on the largest radius of the field of view of the specific objective utilised, and correction factors were introduced to account for possible artefacts introduced by the metallic objectives on the imposed and induced fields. Additionally, the assessment also included the presence of unshielded parasitic electric fields and artificially generated background magnetic fields, which were also combined based on estimated or directly reported values.

3. Results

3.1. Study selection and characteristics

The search strategy performed in this paper was designed to retrieve all papers related to LF MF exposure and calcium homeostasis. The search terms were kept broad for every search engine, which led to the selection of 1717 potential papers in the initial phase (Fig. 1). From these, 1490 articles could already be excluded based on title and abstract, since they did not describe the exposure of cells to magnetic fields. Further investigation of the 227 remaining papers based on full text led to inclusion of 42 studies (McCreary et al., 2006; Conti et al., 1985a; Liburdy, 1992; Galvanovskis et al., 1996; Walleczek and Liburdy, 1990; Lisi et al., 2006; Pilger et al., 2004; Sakurai et al., 2005; Liu et al., 2014; Tonini et al., 2001; Garciasancho et al., 1994; Lee et al., 2002; Hwang et al., 2011; Lindstrom et al., 1995; Nishimura et al., 1999; Grande et al., 1991; Oh et al., 2001; Conti et al., 1985b; Luo et al., 2014; Mattsson et al., 2001; Wey et al., 2000; Lindstrom et al., 1998; Craviso et al., 2003; Fitzsimmons et al., 1994; Fixler et al., 2012; Gaetani et al., 2009; Bernabo et al., 2007; Morabito et al., 2010a; Liburdy et al., 1993; Kim et al., 2013; Piacentini et al., 2008; Loschinger et al., 1999; Yamaguchi et al., 2002; Craviso et al., 2002; Lyle et al., 1997; Coulton and Barker, 1993; Morabito et al., 2010b; McCreary et al., 2002; de Groot et al., 2014; Wei et al., 2014; Wu et al., 2014; Lyle et al., 1991).

The study characteristics of all included papers are shown in Table S1, Supplementary data, including the characteristics of every individual experiment/comparison. These study characteristics varied considerably among the papers included. All in vitro experiments included in our study were performed with cells extracted from

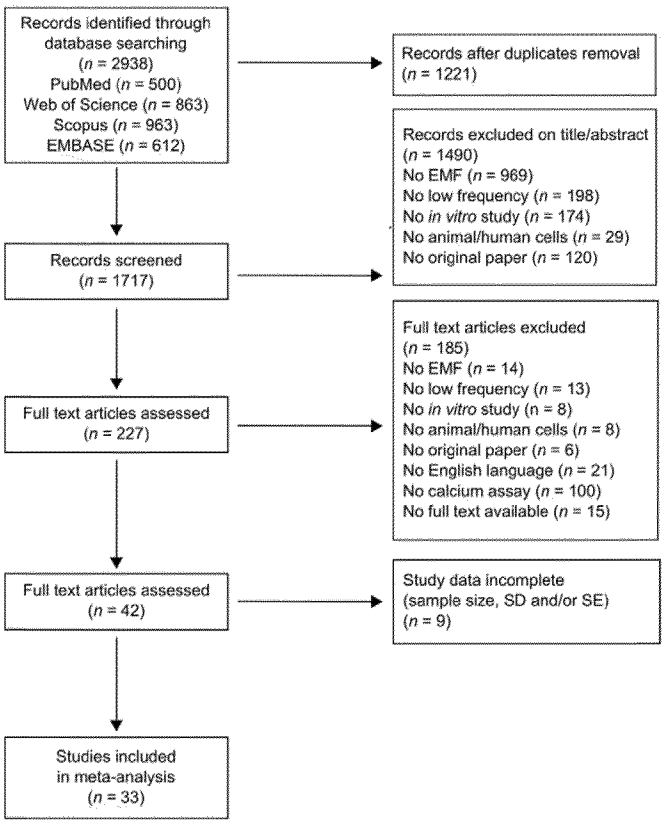

Fig. 1. Flow diagram of the systematic review protocol illustrating the literature research and exclusion process.

mammals, either immortalised cell lines, which were established through isolation of cancer cells or induction of mutations, or primary ex vivo cell cultures. From the 148 experiments, 72 were performed with cell lines and 76 with primary cells. Cells from mice, rats, and humans were the most commonly used; one study each was performed with cells from cows (Craviso et al., 2003) and pigs (Bernabo et al., 2007). The LF MF exposure characteristics were more diverse: the magnetic flux densities ranged from 40 nT to 22 mT, and the duration of exposure ranged from a couple of minutes to many days. In almost two-thirds of the studies (92 of 148), the cells were exposed to 50 or 60 Hz, and only in 15 experiments was a specifically calculated calcium resonance frequency applied. With respect to the timing of exposure, in a little over two-thirds of the experiments (105 of 148) were the measurements of calcium concentration carried out during acute exposure.

To examine the effect of LF MF exposure, all papers compared LF MF-exposed samples to sham exposures; however, the definition of control/sham differed. To minimise sample variation, a sample or cell was used as its own control in 26 of the experiments. In 122 experiments, independent measurements were performed during or after sham or LF MF exposure.

3.2. Reporting quality and risk of bias

The reporting quality of all papers included showed large differences (Fig. 2). While the cell type, duration, frequency, and magnetic flux density were mentioned in all of the papers, in only ca. 30% was the background field during the exposure described. Moreover, 40% did not mention any values for these environmental fields. Furthermore, our risk of bias assessment revealed large or unknown risks. All papers clearly described the frequency and magnetic flux density that was applied to the cells, however only two-thirds (64.3%) described the use of sham exposure or of an unenergised coil for the control conditions. One

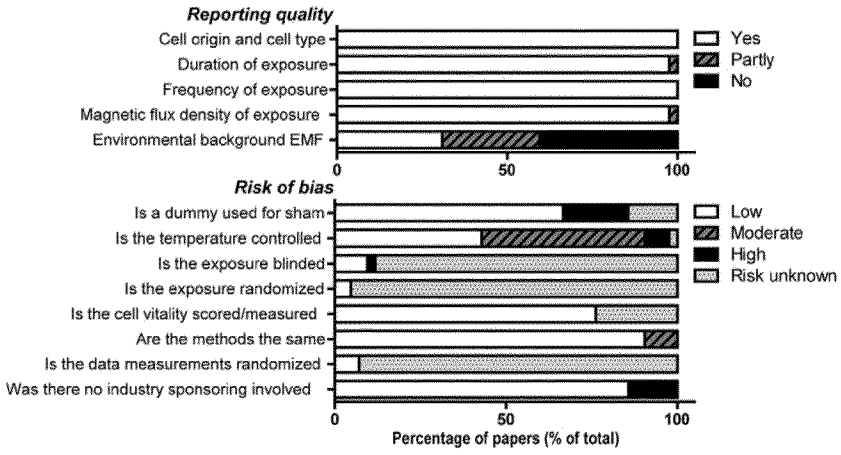

Fig. 2. Reporting quality and risk of bias. Average score in percentage of all 42 papers. For the reporting quality, papers were scored. "Yes", "Partly", or "No" indicates the presence or absence of essential study details. For the risk of bias, a "Low", "Moderate", or "High" risk of bias might be introduced in the studies. When the outcome was not reported or mentioned, the "Risk unknown" was chosen and scored accordingly.

of ten studies lacked description of an identical protocol to measure control and LF MF samples. In 78% of the studies, the vitality of the cells before or after exposure was scored. The largest unknown risks are introduced by unreported blinding of exposure and randomisation of exposure and measurements. Additionally, although temperature control during exposure was described in more than 90% of all papers, in which half also reported the error range, measurements were typically made outside of the culture space, which sets this variable as a possibly important artefact. Regarding industry sponsoring, authors of 6 of the 42 papers reported connections with or employment at a company, which might contribute to the risk of biased outcomes.

3.3. Meta-analysis

3.3.1. Intracellular calcium oscillations

Twelve papers investigating the effect of LF MF exposure on calcium oscillations could be included in our meta-analyses. Four of the eleven papers reported dichotomous outcomes, of which one included dependent measurements. This study was not included in our analysis. The events in the three remaining papers were grouped and odds ratio with a 95% CI was calculated (Supplementary Fig. S1).

In the remaining eight papers, oscillation patterns were measured in 15 experiments, of which only three showed significantly different oscillation patterns compared to control treatment. The overall effect analysis did not indicate a significant effect of exposure (Fig. 3A; SMD - 0.007 [- 0.392, 0.378]; n = 15; s = 5). In two studies that together included seven experiments, the amplitudes of the waves during or after exposure were measured. Three of the seven results indicated a statistically significant decrease in amplitude compared to control conditions. The overall effect of these seven experiments indicated no effect on the amplitude of the calcium oscillations (SMD - 0.994 [- 2.013, 0.024]; n = 7 ;s = 2). The last outcome measured, frequency of the oscillating waves, showed a mixed effect: four experiments showed an increase and two experiments a neutral effect. However, the overall analysis revealed a statistically significant effect of LF MF exposure on the frequency of the calcium oscillations (SMD 1.669 [0.488, 2.849]; n = 6; s = 3).

3.3.2. Intracellular calcium concentration

Reliable measurements of intracellular calcium concentrations can be performed with the stable artificial radioisotope ^{45}Ca, exclusively to measure the entry of calcium from the external medium into the cells. A second approach is the use of chemical fluorescent dyes to quantify the intracellular calcium concentration. These dyes facilitate investigation of potential functions and regulatory mechanisms of calcium in a cell, such as channels and pumps. In 24 of the studies included in our review, the effect of LF MF on the intracellular calcium concentration was investigated with one of the methods described above. We determined the presence of an effect regardless of direction, by expression all differences between control and LF MF exposure as a positive value. This analysis indicates only the presence of an effect and cannot be used to determine the overall direction or effect size. Subsequently, both

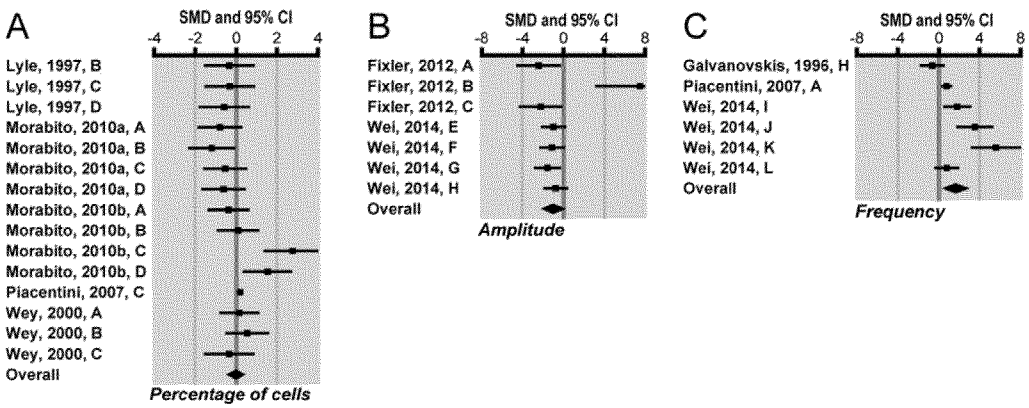

Fig. 3. Influence of LF MF exposure on calcium oscillations. Forest plot for the three different outcomes regarding calcium oscillations. A) The percentage of cells that showed altered oscillation patterns during sham/exposure, B) changes in the frequency, and C) the amplitudes of the calcium waves. The forest plot displays the SMD (squares) and 95% confidence interval of the individual studies. The diamond in each plot indicates the overall estimate and 95% confidence interval.

positive and negative effects were analysed according to the original direction of change. This second analysis was used to estimate the overall direction and effect size. Our review's analysis of intracellular calcium concentrations included 24 studies with 81 experiments. Overall, the presence of an effect of LF MF exposure on intracellular calcium concentration was demonstrated (SMD 0.914 [0.723, 1.104]; n = 81; s = 24) (Fig. 4A). Forty-nine of the eighty-one experiments showed no significant influence of LF MF exposure on the intracellular calcium concentration. In addition, 22 experiments showed an increase in calcium levels, and ten reported a significant reduction in the intracellular calcium concentration. The overall analysis of all 24 papers indicated that the direction of this effect was positive, with a small significant increase in intracellular calcium levels caused by LF MF exposure (SMD 0.351 [0.126, 0.576]; n = 81; s = 24) (Fig. 4b). Heterogeneity was high (I^2 = 83%) and reported standardised mean differences (SMD) ranged from - 10.39 (Bernabo et al., 2007) to 21.62 (Fitzsimmons et al., 1994).

3.3.3. Subgroup meta-analysis of study characteristics

The studies of intracellular calcium concentration included sufficient datasets to perform multiple subgroup comparisons based on characteristics (Table S1) determined a priori. All experiments were combined in subgroups and analysed in two ways. First, we determined whether an effect was present and then provided an estimate of the effect size and direction. In Fig. 5, the effect of the different subgroups shows the effects in one direction. The lower limits of the confidence intervals of all subgroups were above zero, which indicated the presence of an effect of LF MF exposure for every subgroup. However, the direction and magnitude of these effects differed between subgroups (Fig. 6). A large number of fluorescent calcium indicators are available to investigate intracellular calcium concentrations and calcium mobilisation in cells in in vitro studies (Thomas et al., 2000). Intracellular calcium concentration measured with any of the fluorescent calcium dyes showed no significant difference compared to control samples, both during and after exposure (dye-during; SMD 0.137 [- 0.104, 0.378]; n = 23; s = 5; and dye-after; SMD 0.066 [- 0.261, 0.393]; n = 28; s = 13). The isotope ^{45}Ca-studies on the other hand, showed a significant increase in intracellular calcium under the influence of LF MF exposure (^{45}Ca; SMD 1.018 [0.342, 1.694]; n = 30; s = 6), as the confidence interval does not cross the zero-limit that represents the control samples. Comparison of the three techniques (dye before, dye during and ^{45}Ca) revealed no significant difference between the groups.

To examine whether there is evidence for a specific LF MF feature that explains differences in the effect of LF MF exposure reported by different groups, all LF MF exposure characteristics were categorised. The frequency, magnetic flux density, and duration of exposure were studied in subgroup analyses. The magnetic flux density was grouped according to exposure limits for LF MF developed by the International Commission on Non-Ionising Radiation Protection (ICNIRP). Continuous exposure of the human body is allowed magnetic flux densities of up to 200 μT, whereas occupational exposure safety limits are higher, 1 mT (International Commission on Non-Ionizing Radiation, P., 2010). The different magnetic flux densities demonstrated different effects of LF MF exposure. Exposure levels up to 200 μT showed a significant increase of intracellular calcium, while higher exposure levels showed a neutral effect. Comparison of the different subgroups revealed no significant difference among the different exposure levels (b200 μT; SMD 0.612 [0.199, 1.025]; n = 40; s = 8, 200–1000 μT; SMD 0.096 [- 0.280, 0.472]; n = 25; s = 11, and N1000 μT; SMD 0.456 [- 0.119, 1.032]; n = 16; s = 8).

Furthermore, subgroup analysis of the different frequencies indicated that there is a relationship between the frequency applied to the cells and intracellular calcium concentration. The different frequencies were grouped based on two theories present in literature. First, electrical power supplies produce magnetic fields with frequencies of 50 or 60 Hz. These power line frequencies, which are those most often applied in the studies included, did not indicate any effect of LF MF on calcium homeostasis (50/60 Hz; SMD 0.054 [- 0.190, 0.298]; n = 50; s = 19). Another frequency that was investigated extensively during the late 1980s and early 1990s is the ion resonance frequency (IRF) for calcium

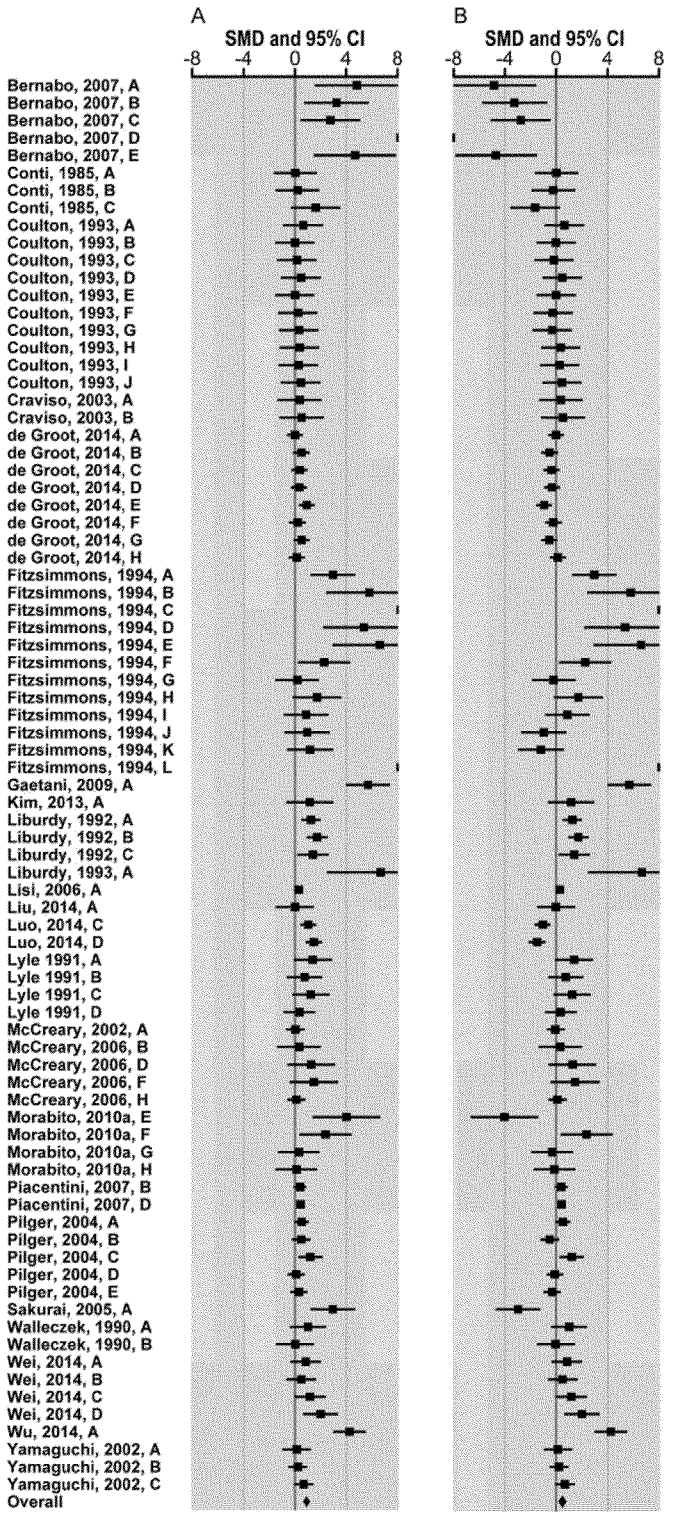

Fig. 4. Influence of LF MF exposure on the intracellular calcium concentration. Forest plot of the 81 studies included that describe intracellular calcium concentrations during or after exposure. A) The presence of an association between LF MF exposure and intracellular calcium concentrations, and B) the direction of the effects. The forest plot displays the SMD (square) and 95% confidence interval (CI) of individual studies. The diamond at the bottom of each graph indicates the overall estimate and 95% confidence interval.

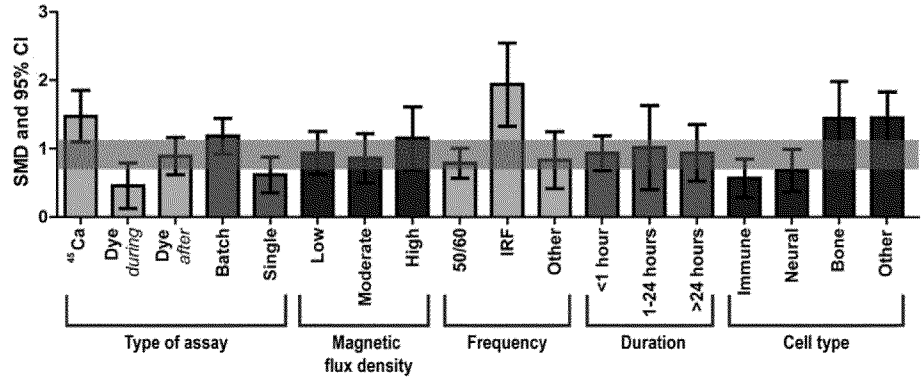

Fig. 5. Subgroup analyses of the different study characteristics to determine the presence of an effect of LF MF on intracellular calcium. The blue horizontal bar shows the overall effect size and 95% confidence interval. Every vertical bar represents the subgroup SMD and 95% confidence interval. All study effects are reported in one direction (positive) to evaluate the presence of an association. Measurements performed with radioactive calcium (^{45}Ca), calcium dyes during acute exposure (Dye during) or after exposure (Dye after) on a batch (Batch) or individual cells (Single), with a magnetic flux density up to 200 μT (Low), 200–1000 μT (Moderate) or above 1000 μT (High). Frequencies were groups based on the frequency, 50/60 Hz (50/60), ion resonance frequency (IRF) and frequencies other (Other) than 50 or 60 Hz or IRF.

ions. The original hypothesis by Liboff (Liboff et al., 1987) suggested that calcium ions are activated by a specific combination of frequency and static (DC) field. In four papers, the existence of the IRF was investigated, and subgroup analysis showed that this relationship might exist, as the subgroup displayed a significant increase in intracellular calcium concentration (IRF; SMD 2.655 [1.293, 4.018]; n = 12; s = 4). A more detailed investigation of the four individual studies included in this subgroup — Coulton and Barker (1993), Lyle et al. (1997), Gaetani et al. (2009) and Fitzsimmons et al. (1994) — showed that only the latter two displayed a significant effect, i.e., very pronounced increase of intracellular calcium induced by LF MF. Interestingly, analysis of papers in which exposure to frequencies other than IRF or 50/60 Hz indicated a small but significant increase of intracellular calcium, although the effect was less pronounced than that evoked by IRFs (Other; SMD 0.205 [0.014, 1.028]; n = 19; s = 5). Only IRFs showed a significant difference compared to the other two subgroups in the analysis.

The last characteristic of the exposure set-up is exposure duration, ranging from a couple of minutes to many days. Subgroup analysis did not show any significant differences for exposures longer than 1 h (1–24 h; SMD - 0.752 [- 1.577, 0.073]; n = 9; s = 2 and N24 h; SMD - 0.046 [- 0.559, 0.467]; n = 17; s = 7). Short exposures up to 1 h on the other hand, indicated a significant increase of cellular calcium (SMD 0.657 [0.374, 0.940]; n = 55; s = 16). This increase after less than 60 min of exposure was significantly different from the negative trend reported after an exposure period up to 24 h. In summary, the magnetic flux density, the frequency applied and the duration of exposure might all be MF characteristics that influence the outcome of the experiments.

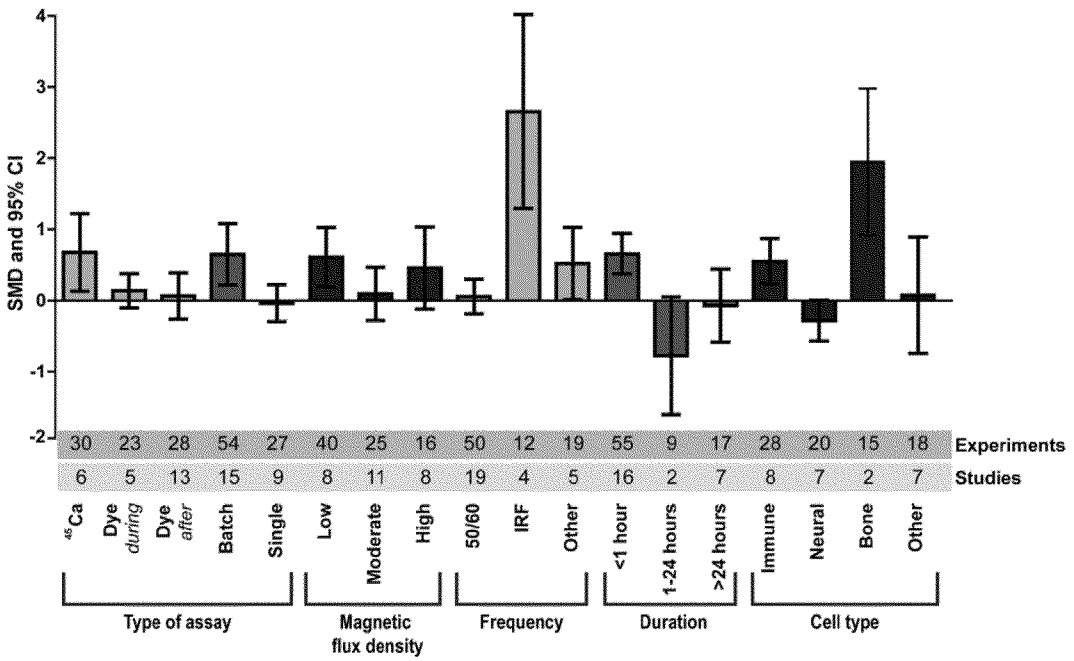

Fig. 6. Subgroup analyses of the different study characteristics to determine the direction of an effect of LF MF on intracellular calcium. All studies were reported with their actual effect size and direction, from which a grouped effect size and direction was calculation. Every vertical bar represents the subgroup SMD and 95% confidence interval. The total number of individual studies and experiments in every subgroup is indicated underneath every bar. Measurements performed with radioactive calcium (^{45}Ca), calcium dyes during acute exposure (Dye during) or after exposure (Dye after) on a batch of cells (Batch) or individual cells (Single), with a magnetic flux density up to 200 μT (Low), 200–1000 μT (Moderate) or above 1000 μT (High). Frequencies were groups based on the frequency, 50/60 Hz (50/60), ion resonance frequency (IRF) and frequencies other (Other) than 50 or 60 Hz or IRF.

The existence of a specific cell type or cellular feature that is susceptible to LF MF exposure is debated in literature (Simkó and Mattsson, 2004; Pall, 2013; Gartzke and Lange, 2002). Our subgroup analysis for the 24 papers reporting intracellular calcium concentration showed significant differences among the different cell types. Immune cells showed an increased intracellular calcium concentration (Immune; SMD 0.543 [0.226, 0.861]; n = 28; s = 8), whereas grouping neural cells showed a neutral effect (Neural; SMD - 0.235 [- 0.521, 0.050]; n = 20; s = 7). Bone-related cell types also indicated significant effects (Bone; SMD 1.921 [0.891, 2.951]; n = 7; s = 2), but caution is required as this subgroup only contained two individual studies. The remaining cell types were grouped as "other" and did not show any effect resulting from LF MF exposure (Other; SMD 0.071 [- 0.744, 0.886]; n = 18; s = 7).

3.3.4. Magnetic and electric field exposure assessment

From the papers included that reported intracellular calcium concentrations determined during or after exposure, the electric and magnetic fields could be calculated (Table S3). These values were plotted as a function of the effect size to investigate a correlation between intracellular calcium and these fields. The total magnetic fields to which the cells are exposed to, are composed of the fields generated by the exposure system (IMF) plus the fields generated by secondary sources (See Supplementary Note 1 for details). A plot of the total magnetic field value as a function of the SMD of intracellular calcium did not show a correlation (Fig. 7A). Furthermore, the calculated IEF values range from 0 to 4.62 V/cm, as cells inside one container experience IEF of different strengths, depending on the distance from the centre and the orientation of the magnetic field. We also plotted the maximum electric fields that are generated by the IMF plus the field generated by secondary sources as a function of the SMD and observed no correlation (Fig. 7B). The calculated total electric field was higher in most experiments, as the most significant possible secondary source corresponds to parasitic electric fields. These fields are generated by a potential drop along the inductance of the coils of the exposure system (Schuderer et al., 2004) and were found to be typically unaccounted for in the literature reviewed. Since these could contribute to the total electric field in the cultures, we also explored the possibility of the presence of parasitic electric fields. The line drawn in Fig. 7b indicates that the effect size potentially correlates to the total electric fields, based on the assumption of a linear relationship between the two variables, although the correlation is very weak (R^2 = 0.096 ± 0.037).

3.3.5. Sensitivity analysis

A sensitivity analysis is used to assess the robustness of the results of a meta-analysis. Exclusion of the studies in which mathematical calculations were made to estimate the sample size did not influence the effect size of the overall analysis of intracellular calcium concentrations, an indication that our estimates are robust.

3.3.6. Publication bias

In Fig. 8, a funnel plot generated from the data in all 24 papers that described intracellular calcium measurements is displayed. A trim and fill algorithm (Duval and Tweedie, 2000) was used to estimate the number of studies missing to be four. Addition of these four missing studies indicates only a small overestimation of the effect size.

4. Discussion

An accumulating number of papers that report contradicting health effects induced by LF MF exposure have been published. This has led to an even larger increase in papers published that aim to investigate possible molecular mechanisms to explain these effects. This systematic review and the meta-analyses included, focus on the modulation of calcium homeostasis by LF MF exposure in in vitro model systems. These meta-analyses revealed an association of LF MF exposure with increased frequency of inherent oscillations of cellular calcium concentrations. A positive association could also be observed for overall intracellular calcium. However, the effect size and direction of every effect only indicated significant effects for experiments measured with radioactive calcium, experiments that applied frequencies other than 50 or 60 Hz or with weak magnetic flux density. In addition, experiments that involved either a short exposure, or were performed on immune or bone or a batch of cells, showed effects after exposure. Some of these results might be due to the inclusion of three papers that reported extremely large effects of LF MF exposure (Fitzsimmons et al., 1994; Bernabo et al., 2007; Liburdy et al., 1993).

Calcium oscillations represent a naturally occurring process that plays a vital role in intracellular signalling (Berridge et al., 2003). These short cytoplasmic waves in calcium concentrations are a common phenomenon in both excitable cells, such as cardio-myocytes and neural cells (Wu et al., 2010; Wang and Gruenstein, 1997), as well as in non-excitable cells, like immune, endocrine, and endothelial cells (Song et al., 2012). Moreover, calcium oscillations regulate the activation of intracellular proteins (Smedler and Uhlen, 2014). Calcium

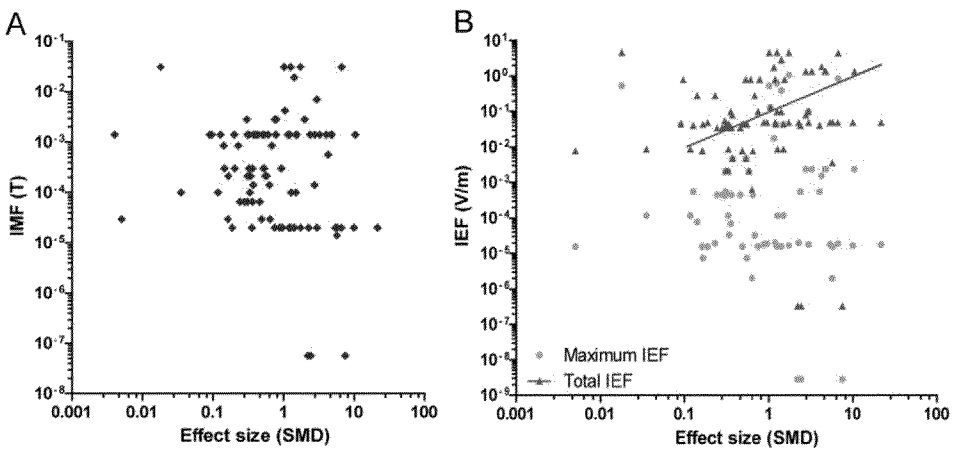

Fig. 7. Regression plot of the effect sizes versus magnetic and electric fields in the culture space. The calculated effect size (SMD) of every intracellular calcium measurement was plotted against A) the imposed magnetic field and B) maximum (blue) and total (red) induced electric field (IEF). The term "maximum IEF" refers to the IEF on the largest radius of conductive liquid on the culture space perpendicular to the homogeneous IMF. The term "Total IEF" refers to the summation of the "maximum IEF" and the upper boundary found for the possible parasitic electric fields (assuming concurrence of direction). Linear regression analysis (red line) was performed for the latter.

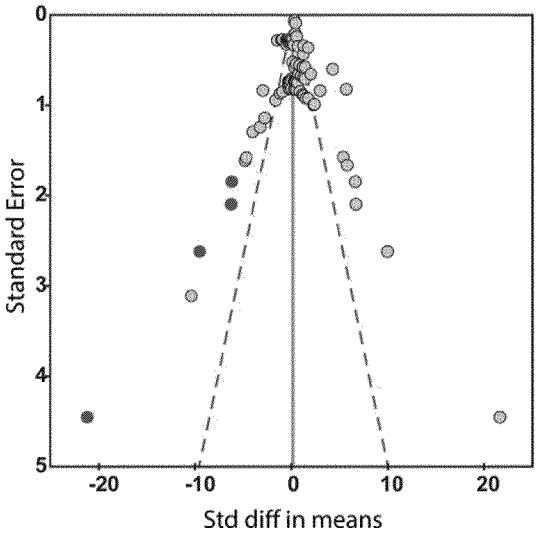

Fig. 8. Funnel plot. All 81 individual studies (grey circles) that measured intracellular calcium during or after exposure were plotted according to their standard error and standard difference in means (SMD). The 95% confidence interval (dotted red lines) of the overall effect (grey line) is adjusted by the addition of four studies (red circles) and gives a new estimated effect (vertical red line).

oscillations are decoded by one or several intracellular molecules that sense the calcium concentration and modulate their activity accordingly. The different outcome measures of calcium oscillation in our study revealed a heterogeneous effect of LF MF exposure. The percentage of cells with oscillating calcium concentrations during or after exposure did not change and the amplitude of oscillations remained unaffected. The frequency of the waves on the other hand, was significantly increased. Since the frequency of calcium oscillations determines which downstream proteins are activated (Smedler and Uhlen, 2014), these results suggest that LF MF exposure may modulate cellular behaviour. Only one of the included studies reported both frequency and amplitude as a study outcome, with effects similar to our estimates (Wei et al., 2014), i.e., increased frequency in combination with reduced amplitude. Unfortunately, downstream effects of altered oscillations after LF MF exposure were not investigated in this paper. It has been demonstrated in the recent literature that altered cellular behaviour, regulated by the frequency of calcium oscillations, applies only in situations where the amplitude remains constant (Song et al., 2012). Since frequency, amplitude, and duration of oscillations together determine the ultimate intracellular signalling, we are only able to show the association, but are not able to predict the potential downstream effects of LF MF exposure.

Calcium oscillations regulate protein activity and gene expression upon stimulation (Song et al., 2012), but in resting cells, a stable intracellular calcium concentration is maintained. Cytoplasmic calcium is sequestered mainly in calcium stores, like the endoplasmic reticulum. Moreover, sodium-calcium exchangers and/or plasma membrane calcium ATPases (PMCA) pump cytoplasmic calcium back into the extracellular milieu with no need for biological stimuli (Berridge et al., 2003). Our meta-analysis showed an effect of LF MF exposure on the intracellular calcium concentration. This should be interpreted with some caution, since the directions of the reported effects differed between studies. Only 25% of the studies reported a significant increase of calcium, whereas 12.5% reported decreased concentrations. Moreover, the overall effect size found was quite small. This suggests that either the effect of LF MF depends on experimental conditions, or that uncontrolled confounding variables influence the outcomes of the studies (McCreary et al., 2002; Portelli et al., 2013; Butler et al., 2013).

Subgroup analysis of the three different methods of analysis for intracellular calcium showed that only the experiments performed with radioactive calcium indicate a positive association. Measurements performed with a fluorescent dye, during or after exposure, showed a neutral effect. Based on the ^{45}Ca-measurements, we could speculate that LF MF exposure influences the calcium homeostasis by modulating the calcium uptake or efflux. In accordance with the present results, it has been reported that LF MF exposure induces an increase in inositol 1,4,5-triphosphate (IP3) levels (Korzhsleptsovaet al., 1995), an increase in protein kinase C (PKC) activity, or modified activity of voltage-gated channels (Pall, 2013; Piacentini et al., 2008). All these processes could lead to an altered calcium homeostasis. However, the effects might be compensated by calcium efflux or uptake in intracellular stores, a hypothesis that is supported by the calcium-dye subgroup, in which intracellular calcium was measured after exposure. The cells in these studies maintained or restored the intracellular calcium balance after an initial increase, regardless of continuous exposure. Though, if the influx or efflux of calcium changed during LF MF exposure, this implies that a transient difference in the intracellular calcium concentration would be measured, just before the cells readjust their internal balance. Nevertheless, meta-analysis of the papers in which the calcium-dye method was used to measure calcium concentration during exposure did not substantiate this hypothesis. This contradiction does not strengthen the association found in our study and points to conflicting outcomes induced by uncontrolled technical parameters, such as temperature (Butler et al., 2013) or background fields (Portelli et al., 2013).

The study characteristics of the papers reporting intracellular calcium concentrations enabled us to investigate the potential presence of specific LF MF exposure features that may be related to the biological effects of exposure. Therefore, subgroup analyses were performed based on frequency, magnetic flux density, or exposure duration. The existence of a frequency window, which modulates cellular signalling, has been debated (Niu et al., 2003; Pilla et al., 1999). However regression plots of all studies did not reveal a specific frequency window (data not shown). The calcium IRF has been hypothesised to move calcium ions in a cell, and our meta-analysis showed significant increase of intracellular calcium. This subgroup differed significantly from the other two clustered frequencies. A more detailed investigation of the four individual studies included in the IRF-subgroup revealed that the results are not univocal. Only a few experiments reported a pronounced increase of intracellular calcium by LF MF exposure, whereas neutral effects were reported from other experiments. Power line frequencies of 50 or 60 Hz were reported in 50 of the 81 included studies, and no significant calcium modulation by LF MF was noted. Interestingly, the use of frequencies other than IRF or 50 or 60 Hz also indicated LF MF-induced increases in intracellular calcium, with frequencies ranging from 16 to 120 Hz. These results indicate that the universal exposure frequencies of 50 and 60 Hz in our daily lives might not impact possible health effects. Rather, an association with other less common frequencies could exist. Moreover cells might adapt to constant environmental exposure. This second notion is supported by Goodman et al. (1992) and Lin et al. (1996), both of whom showed that chronic exposure of cellular systems could lead to adaptation without the occurrence of any effects in vitro. We assessed a similar effect for the exposure duration. Our meta-analysis indicated a significant increase in intracellular calcium concentrations when cells were exposed for no longer than 60 min. Increasing the exposure duration from 1 h up to 24 h led to a not significant trend of lower intracellular calcium levels. Based on this subgroup meta-analysis, we could hypothesise that the biological effect of LF MF depends on the duration of exposure. An initial increase in intracellular calcium by influx or reduced efflux is followed by a period to re-establish the homeostasis after 1 hour. Eventually the cells return to a state of balanced calcium levels and are no longer affected by any exposure.

Biological effects that depend on the strength of the magnetic flux density have been advocated, each with their own threshold (Blackman et al., 1993; Zhang et al., 2006). We found an indication for increases in intracellular calcium levels in response to LF MF exposure.

These were related only to magnetic flux densities of up to 200 μT. An association with low level fields could potentially be interpreted as a health risk in normal daily life. However, this specific subgroup contained one study published by Fitzsimmons et al. (1994) with extremely high effects at 40 nT LF MFs, while the other studies showed consistent effects with neutral or only minor differences. A flux density of 40 nT is very low, but still too high to represent average daily exposure (van Tongeren, 2004; Bolte et al., 2015). However, such magnitudes could in fact be easily obscured by artefacts introduced by unaccounted secondary sources (see Supplementary Fig. S2). For these reasons, this subgroup meta-analysis should be interpreted with caution, because the associations might be related to only one included study that influences our estimates. In summary, there are indications that low level fields with a specific frequency and short duration influence calcium homeostasis of cells in an in vitro model system. However these results are not unequivocal. A considerable proportion of studies also contradict the association, indicating that the evidence of association is weak.

Selective LF MF sensitivity of different cell types has been postulated. The central nervous system for instance, might be particularly vulnerable since neural function is highly charge-dependent (Pall, 2013; de Groot et al., 2014). Immune cells on the other hand, produce free radicals, the generation of which has been proposed to be sensitive to low-levels of magnetic fields (Simkó and Mattsson, 2004). Furthermore, bone cells not only use calcium as an intracellular second messenger, but also convert calcium ions into a solid extracellular matrix during cartilage and bone production (Meghji, 1992). With our subgroup analysis, we found significant evidence for a cell-type related effect. The intracellular calcium concentration of immune and bone-related cells was increased during or after LF MF exposure. Additionally, neural cells showed a neutral effect with possible decrease in intracellular calcium. These contradictory trends indicate that a possible effect of LF MF exposure might indeed be related to the type of cells. This could explain why prominent effects in multiple other cell types were found, even though the direction and effect size are not significant. We grouped cells with different phenotypes together, but multiple biological targets of LF MF might exist, that give rise to different interactions with an overall neutral effect. If LF MF interact with different biological targets, an overall comparison of all in vitro studies could be less reliable. These results emphasise the importance of investigating different cells types without generalising them in in vitro and in vivo experiments regarding the mechanism of LF MF exposure.

Finally, we examined if the measured effect sizes could be related to the magnetic and electric fields in culture. To be able to back-calculate these fields for most of these studies, assumptions should be made to allow reconstruction of the experimental conditions from the information provided in the papers. Errors in the reporting of the physical parameters were commonplace, such as diameter reported as radius, or μT instead of mT. Some of these discrepancies were detectable and conciliation was possible, sometimes with the insertion of uncertainty; others were incomplete beyond reasonable estimation and could not be included in this study. This highlights the importance of thorough description of the experimental setup in reporting. Furthermore, we calculated the maximum fields that cells could experience inside cell culture incubators or under a microscope. In the case of exposures under microscopes, it is generally assumed that the IEF is around zero under homogeneous magnetic fields, as the imaging area is in the middle of the magnetic vortex. However, modelling of inhomogeneity in the IMF induced by the presence of microscope parts in close proximity to the culture volume has been shown to introduce significant variations to the maximum IEF (around 37%), spatial gradients of the IMF (N200%), and dislocation of the vortex (Chatterjee et al., 2001). The ranges provided for the magnitude and direction of electric fields in this study correspond to the best-informed estimate possible, however real values are case specific and may differ significantly within the ranges provided or calculated. Ideally, the total electric field in the culture volume must be measured directly, as it comprises the summation of all sources of electric fields and magnetic fields in proximity with the culture container.

Although exposures were catalogued and compared for the maximum possible fields in the culture space, the strength of electric and magnetic fields depend on the location of the cells within the culture container and the orientation of the magnetic field. For parallel IMF, most of the adhered cells on the culture plane are exposed to homogeneous fields (Bassen et al., 1992). However, when the imposed homogeneous magnetic field is perpendicular to the culture plane, the IEF vortex will be located at the centre of the culture surface and its magnitude will grow linearly as a function of the radius (Liburdy, 1992). As a consequence, about 50% of the cells are exposed to electric fields that range from 0% to 70% of the maximum IEF on the container (Bassen et al., 1992). Besides this variability in exposure, significant uncertainty is generated with cells in suspension, as these are free to move within different exposure levels. Consequently, the power of this assessment is limited, as sufficient countermeasures for such heterogeneity in exposure were not employed in most experimental designs.

We did not observe a correlation for the IMF or maximum IEF, but found a weak association with the total IEF. Since the total IEFs took into account the maximum levels of parasitic electric fields possible, and IEFs alone did not indicate a relation, we hypothesise that parasitic fields and other unaccounted parameters of the exposure systems may have significantly influenced the experimental outcomes. These parameters need to be controlled in future experiments to ensure that conclusions are based on repeatable LF MF exposures.

Publication biases are an unavoidable part of a systematic review and meta-analysis, but the large number of neutral effects included in our analyses already indicated that this type of bias is less pronounced in LF MF research. Neutral data published on LF MF exposures are valuable and could reduce societal concern regarding the potential health effects of LF MF (Siegrist and Cvetkovich, 2001). The funnel plot of our largest data set (Fig. 8) indicates that four papers might be missing from our intracellular calcium analysis, confirming that this type of bias did not strongly affect our overall analysis. However, in our analysis, we did not correlate our findings with the impact factor of the journals: neutral data might be more likely published in low-impact journals, whereas results of significant differences are more likely to be published in higher-impact journals, which would qualify as a type of publication bias. However, funnel plot in Fig. 8 indicates that our meta-analysis outcomes are not influenced by the absence of a small number of papers.

Performing a meta-analysis of in vitro studies could lead to heterogeneity, due to the numerous different cell types, assays, and culture conditions explored in in vitro model systems. Our meta-analysis showed a high heterogeneity (I^2 N 75%), which might not only be caused by the variety of cell types and cell origins studied but also by variation in exposure characteristics such as frequency, magnetic flux density, and exposure duration. However, grouping similar papers did not reduce heterogeneity. This is a limitation that necessitates careful interpretation of every meta-analysis. One of the few systematic reviews that also combined experiments performed with in vitro cell cultures and LF MF exposure presented a similar heterogeneity; I^2 N 88% (Adams et al., 2014). After heterogeneity, a substantial risk of bias was introduced by a lack of blinding, temperature control, or cell viability measurements. There is no gold standard for in vitro experiments, but these factors could confound the outcome (Butler et al., 2013; Blackman et al., 1991). Information concerning the use of an identical exposure system for sham treatment was lacking, and background fields during exposure were poorly described (Portelli et al., 2013). Owing to the lack of these crucial components in design and reporting quality, results should always be interpreted with caution.

For future research regarding the effects of LF MF exposure, it is important to confirm the positive association with intracellular cellular measurements performed with radioactive calcium. Our meta-analysis

indicated a positive association, however exacting replication of the ^{45}Ca experiments would confirm and strengthen the association or emphasise the presence of outlier studies in literature that conceal the true effect. Our subgroup analyses also indicated a possible interaction related to the use of uncommon frequencies, with low magnetic flux densities, for short exposure periods. A combination of all these features presents a good experimental design for future research. Technical bias can be minimised through independent collaboration between different research groups. Furthermore, the calcium oscillation experiments revealed a mixed effect that depended on the outcome variable; investigation of such effects in more detail in an experimental setup that simultaneously measures frequency, amplitude, calcium content, and, preferably, downstream protein activation during LF MF exposure is advised.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2016.01.014 .

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Supplementary Note 1

Imposed magnetic field (IMF) and induced electric field (IEF) exposure assessments

A thorough survey of the exposure conditions reported in the literature was performed to extract all information of relevance regarding the electric and magnetic field exposure parameters in the culture space. Only publications that provided enough explicit or implicit information about their exposure conditions were included in this study.

Signal type: The maximum dB/dt presented by each specific signal was utilised for the calculation of the maximum induced electric field in the culture space. For all continuous signals, the maximum dB/dt was extracted from the dominant harmonic in the signal. In the case of intermittent signals, the maximum dB/dt resulting from the modulation of the continuous signal was extracted. Intermittent signals that produced modulations with frequency components smaller than the frequency components of the carrier signal ("soft switching") were treated as contin uous signals. In the case of sinusoidal signals explicitly generated by a dedicated function generator or "synthesi sed", the contribution of higher harmonics other than the fundamental frequency was deemed irrelevant. Signals were considered "generic power signals" in cases w here versions of the local power distribution system (step-down transformer, etc.) or where information about its higher harmonics was not provided. The definition o f "generic power signals" utilis ed in this study corresponds to the maximum accepted distortion for low- to medium-voltage power systems by the International Electrotechnical Commision (IEC) [1996], which is comprised of a multiple-harmonic signal requiring the consideration of frequency-weighed parameters for the calculation of the maximum electric fields induced in the cell medium. For the calculations here presented, the parameters utilised for homogeneous magnetic field exposure are given in [70]. In the cases where the generation of such sinusoidal fields was not expli citly specified, both "synthesised" and "generic power signal" categories were assumed. Studies in which non-sinusoidal signals were utilised were included only when the maximum dB/dt of the signal utilised was specified.

Signal magnitude: The applied magnetic field magnitude reported in the publications reviewed is here and throughout the text reported as "peak" magnitude, that is, one-half of the "peak-to-peak" difference in the waveform. In the cases where the magnitude of the reported signals was not explicitly specified, both root-m ean-square (RMS) and "peak" value s were assumed.

Signal spatial distribution: The homogeneity of the imposed magnetic field (IMF) is highly dependent on the geometric configuration of and details regarding the exposure system. Some of the publications reviewed provided information about the homogeneity of the IMF over the culture volume assessed with direct measurements. However, most of the magnetic field sensors utilised were comparable in size to the exposure coil systems or to the culture containers. Furthermore, measurement resolution on the exposed area was lacking in many studies, which raises questions about the ability of such measurements to detect possible gradients generated either by the coil systems or by extra metallic features of the exposure

systems (microscope plates, objectives, etc.) [86, 91]. For this reason, a measure of the deviation of the reported IMF magnitude was obtained for each exposure configuration in the reviewed literature (usually based on calculations, measurements or estimations at the centre of the exposure system or culture container) and is reported as multipliers (max and min) for the worst-case inhomogeneity on each exposure system.

The homogeneity of the IMF was calculated for each exposure system and its associated culture container over the volume of interest taking into consideration their mutual relative positions. For commonly used configurations, i.e., round and square single and Helmholtz coils, the results for these calculations are tabulated [92]. However, for other configurations based in round coils (off-centre plane coils and solenoids, finite solenoids, multiple coil systems) routines were built in MATLAB R2007a software (Mathworks, Natick, MA) and validated with the on-axes solutions as these require off-axis calculations involve elliptical integrals [93]. The maximum variation of the magnetic field magnitude over the plane of greater area perpendicular to the imposed magnetic field referenced to the value at the centre of the culture container was extracted and is reported as a multiplier in Table S3 (available upon request). The calculation was performed for the designated culture space described for each experiment. In the cases were this space was not explicitly described, estimations were made based on the dimensions and specificities of the exposure systems and their associated culture containers. For arrangements of discrete coils and solenoids, homogeneity was assessed inside a coaxial cylinder centred on the exposure location with radius equal to the larger dimension between the radius of the culture container collective volume escribed sphere and 60% of the coil radius (default), and a height equal to the larger dimension between the culture containers collective wet volume and 10% of the coil radius (default). For the known configurations of Helmholtz, Merritt, and Maxwell coils, etc., homogeneity was assessed inside a concentric sphere centred on the culture container collective volume with radius equal to the larger dimension between the radius of the culture container collective volume escribed sphere and 60% of the greatest coil radius (default). Default values were assumed in cases where the culture container collective volume was not explicitly specified. Default culture container sizes were also assigned in cases where the culture container sizes were not explicitly specified. The effect of microscope-induced spatial inhomogeneity was considered by multiplying the IMF signal magnitude of such exposure systems by a factor of 2.33 to account for possible asymmetric distortions attributable to commonly utilised parts like springs, screws, studs with nickel-chrome based coatings and iron internal components of microscope objectives and other components. Distortions observed could be several millimetres away from the focal plane and detectable only partially by direct measurements, as the gradients generated are often too sharp for conventionally-sized measurement equipment [91]. All publications report (or imply) the generation of linearly polarised imposed magnetic field.

Perpendicular plane maximum radius: The radius representing every culture container corresponds to the radius of the maximum inscribed circle on the plane of greatest area perpendicular to the imposed magnetic field on the culture volume. Taking the surface of the culture liquid as the reference, the plane of greatest area could then be limited entirely by the

container in the case of "perpendicular (⊢)" orientations , e.g. , a Petri dish exposed to magnetic fields applied perpendicular to the plane of the culture, or by the container and the liquid surface in the case of "parallel (∥)" orientations , e.g., a Petri dish exposed to magnetic fields applied parallel to the plane of the culture. For the cases in which the height of the liquid was not explicitly described for conventional culture containers, the height was taken from the maximum standard culture liquid volume to surface area (0.5 ml/cm ^{2}) that allows the minimum gaseous diffusion of oxygen required by most cells [94]; for cells under coverslips, the height was set to 0.1 mm from the standard height of the counting chamber of a Neubauer hemocytometer [94]. The radius corresponding to the area observed under 20×, 40 ×, and 100, objectives was also utilised for IEF calculation in the case of cultures for which biological effects were recorded while exposed under the microscope for magnetic fields applied perpendicular to the culture/observation plane (in the case of parallel orientations, the radius would correspond to the height of the liquid instead).

Induced electric field calculation : Upper boundaries for the induced electric fields were obtained from a simplified form of the Maxwell-Faraday equation, in which the magnitude and direction homogeneity of the IMF over the culture volume is assumed. Maximum and minimum induced electric fields were calculated based on an assumption of the most extreme combination of exposure parameters for this expression. In the case of exposures under a microscope, calculations were also made based on the largest radius of the field of view of the specific objective utilised.

Extra electric and magnetic field artefacts : The magnitude of the contribution to the total induced electric field on the culture space was assessed for secondary electric and magnetic field sources. Artefacts other than the distortion imposed by metallic hardware close to the culture space that may have a significant influence in the resultant fields were also considered:

a) **Artificially-generated time-varying background magnetic fields:** The total IMF to which the culture space is exposed is composed of the field generated by the exposure system plus that of generated by secondary sources (electrically heated microscope stages, laboratory equipment, adjacent power distribution lines, Incubation systems, etc.). Magnitudes for the secondary sources were obtained from the reviewed literature when available. In the case this information was not available, 240uT was used instead as an upper boundary according to previously surveyed data on biological incubators [75].
b) **Parasitic electric fields:** The total electric field in the culture space is composed of the IEF by the IMF and also of parasitic electric fields. The latter are generated in the surrounding space by the potential drop along the inductance of the coils of the exposure system [70], the contribution of which to the total electric field in culture could be relevant depending on the specific special distribution and magnitude. Magnitudes for the parasitic electric fields were obtained from the reviewed literature when available. For cases where these values were not provided, an estimation was made by dividing the potential drop reported by the diameter of the coil system escribed sphere when the feed point of the coil system was not explicitly described . In cases where the potential drop was not provided, it was calculated via Ohms law. For

this, the coil inductive reactance and resistance were utilised to calculate the total impedance, which was then multiplied by the current injected into the coil system. In cases were the wire material was not specified, Cu ($\varsigma = 5.96 \times 10^{-7}$ S/m) was assumed. In cases where the wire diameter was not specified and could not be deduced from other provided parameters, it was assumed that 28 AWG, 0.32 mm diameter was used. Coil inductances were calculated according to [ARRL] [95] for the cases where direct measurements were not provided, and single-layer coil configurations were assumed for the cases in which the coil's system geometry was not sufficiently described. In cases where the height of the coil was not specified, 0.5 cm/100 turns of wire was assumed. In cases where the injected current was not specified, it was calculated depending on the mechanical specifications of each coil system [96] and the maximum IMF generated by the system obtained as described previously in this section. Finally, an estimate of the upper boundary for the parasitic electric field inside the medium ($\varepsilon_r = 80$) on the culture containers was calculated on the basis of the maximum possible parasitic electric field obtained. A shielding factor of 300 was applied in cases where shielding was used, but measurements of residual fields were not provided [70].

Table S1. Summary of published studies considered in the meta-analysis; Origin (O): EV = *ex vivo*, CL = cell line; reported magnetic flux density (MFD); exposure moment (E): D = during data measurements, P = pre-exposure; Control group (Con): Own = dependent measurement with a cell being its own control situation, Two = two independent groups for control/sham and MF; assay type (A): Co = calcium oscillations, Ic = intracellular; Batch/Single (B): S = single cell measurement, B = batch or group of cells measurement; Stimulation (Stim): Yes = stimuli applied to provoke calcium influx

Reference		Cell description	O	Cell Type	Freq	MFD	Duration	E	Con	Chemicals	A	Outcome unit	B	Stim
Mattsson, 2001	A	Jurkat E6-1, source 1	CL	Immune	50 Hz	0.15 mT	7 min	D	Own	Dye-Fura2	Co	Events	S	No
Mattsson, 2001	B	Jurkat E6-1, source 2	CL	Immune	50 Hz	0.15 mT	7 min	D	Own	Dye-Fura2	Co	Events	S	No
Mattsson, 2001	C	Jurkat E6-1, source 3	CL	Immune	50 Hz	0.15 mT	7 min	D	Own	Dye-Fura2	Co	Events	S	No
Wey, 2000	A	Jurkat E6-1	CL	Immune	50 Hz	0.15 mT	4 min	D	Two	Dye-Fura2	Co	% of total	S	No
Wey, 2000	B	Jurkat E6-1	CL	Immune	50 Hz	0.15 mT	4 min	D	Two	Dye-Fura2	Co	% of total	S	No
Wey, 2000	C	Jurkat E6-1	CL	Immune	50 Hz	0.15 mT	4 min	D	Two	Dye-Fura2	Co	% of total	S	No
Conti, 1985a	A	PBMCs	EV	Immune	3 Hz	6 mT	72 h	P	Two	45Ca	Ic	CMP	B	No
Conti, 1985a	B	PBMCs	EV	Immune	3 Hz	6 mT	72 h	P	Two	45Ca	Ic	CMP	B	Yes, PHA
Conti, 1985a	C	PBMCs	EV	Immune	3 Hz	6 mT	72 h	P	Two	45Ca	Ic	CMP	B	Yes, PMA
Lindström, 1998	A	Jurkat E6.1 (Wildtype)	CL	Immune	50 Hz	0.15 mT	8 min	D	Own	Dye-Fura2	Co	Events	S	No
Lindström, 1998	B	Jurkat JRT.T3.5	CL	Immune	50 Hz	0.15 mT	8 min	D	Own	Dye-Fura2	Co	Events	S	No
Lindström, 1998	C	Jurkat J.CaM1/rep3	CL	Immune	50 Hz	0.15 mT	8 min	D	Own	Dye-Fura2	Co	Events	S	No
Lindström, 1998	D	Jurkat J.CaM1/lck	CL	Immune	50 Hz	0.15 mT	8 min	D	Own	Dye-Fura2	Co	Events	S	No
Tonini, 2001*	-	NG108-15	CL	Neural	50 Hz	120 μT 360 μT	100 sec	D	Own	Dye-Indo1	Ic	RFU	S	No
Liburdy, 1992	A	Rat thymic lymphocytes	EV	Immune	60 Hz	22 mT	60 min	D	Two	45Ca	Ic	CMP	B	Yes, ConA
Liburdy, 1992	B	Rat thymic lymphocytes	EV	Immune	60 Hz	22 mT	60 min	D	Two	45Ca	Ic	CMP	B	Yes, ConA
Liburdy, 1992	C	Rat thymic lymphocytes	EV	Immune	60 Hz	22 mT	60 min	D	Two	45Ca	Ic	CMP	B	Yes, ConA
Craviso, 2003	A	Chromaffin cells	EV	Neuro-endocrine	60 Hz	1 mT	15 min	D	Two	45Ca	Ic	CMP	B	No
Craviso, 2003	B	Chromaffin cells	EV	Neuro-endocrine	60 Hz	1 mT	15 min	D	Two	45Ca	Ic	CMP	B	No

Fitzsimmons, 1994	A	TE-85	CL	Bone	15.3 Hz IRF	60 µT	30 min	D	Two	45Ca	Ic	CMP	B	No
Fitzsimmons, 1994	B	SaOS-2 ALP low	CL	Bone	14.3 Hz	60 µT	30 min	D	Two	45Ca	Ic	CMP	B	No
Fitzsimmons, 1994	C	SaOS-2 ALP low	CL	Bone	15.3 Hz IRF	60 µT	30 min	D	Two	45Ca	Ic	CMP	B	No
Fitzsimmons, 1994	D	SaOS-2 ALP low	CL	Bone	16.3 Hz IRF	60 µT	30 min	D	Two	45Ca	Ic	CMP	B	No
Fitzsimmons, 1994	E	SaOS-2 ALP low	CL	Bone	17.3 Hz	60 µT	30 min	D	Two	45Ca	Ic	CMP	B	No
Fitzsimmons, 1994	F	SaOS-2 ALP low	CL	Bone	18.3 Hz	60 µT	30 min	D	Two	45Ca	Ic	CMP	B	No
Fitzsimmons, 1994	G	SaOS-2 ALP high	CL	Bone	14.3 Hz	60 µT	30 min	D	Two	45Ca	Ic	CMP	B	No
Fitzsimmons, 1994	H	SaOS-2 ALP high	CL	Bone	15.3 Hz IRF	60 µT	30 min	D	Two	45Ca	Ic	CMP	B	No
Fitzsimmons, 1994	I	SaOS-2 ALP high	CL	Bone	16.3 Hz IRF	60 µT	30 min	D	Two	45Ca	Ic	CMP	B	No
Fitzsimmons, 1994	J	SaOS-2 ALP high	CL	Bone	17.3 Hz	60 µT	30 min	D	Two	45Ca	Ic	CMP	B	No
Fitzsimmons, 1994	K	SaOS-2 ALP high	CL	Bone	18.3 Hz	60 µT	30 min	D	Two	45Ca	Ic	CMP	B	No
Fitzsimmons, 1994	L	TE-85	CL	Bone	16.3 Hz IRF	60 µT	40 min	D	Two	45Ca	Ic	CMP	B	No
Fixler, 2012	A	Cardio-myocytes	EV	Muscle	15 Hz	40 nT	30 min	D	Own	Dye-Indo1	Co	Amplitude	S	No
Fixler, 2012	B	Cardio-myocytes	EV	Muscle	16 Hz	40 nT	30 min	D	Own	Dye-Indo1	Co	Amplitude	S	No
Fixler, 2012	C	Cardio-myocytes	EV	Muscle	17 Hz	40 nT	30 min	D	Own	Dye-Indo1	Co	Amplitude	S	No
Sakurai, 2005	A	HIT-T15	CL	Epithelial	60 Hz	5 mT	30 min	P	Two	Dye-Fluo3	Ic	Ratio F/F0	B	Yes, glucose
Sakurai, 2005*	B	HIT-T15	CL	Epithelial	60 Hz	5 mT	30 min	P	Two	Dye-Fluo3	Ic	Ratio F/F0	B	No
Gaetani, 2009	A	Cardiophere-derived cells	EV	Epithelial	7 Hz IRF	2.5 µT	5 d	P	Two	Dye-Oregon Green	Ic	RFU	S	No
Oh, 2001*	-	HEL299 and Jurkat	CL	Epithelial Immune	55-60 Hz	1 mT	24 h	P	Two	Dye-Indo1	Ic	RFU	B	No
Conti, 1985*	-	Lymphocytes	EV	Immune	3 Hz	2.3 mT 6.5 mT	1 h	P	Two	45Ca	Ic	CMP	B	No
Bernabo, 2007	A	Spermatozoa	EV	Reproductive	50 Hz	1 mT	2 h	P	Two	Dye-Fluo3	Ic	nM	B	No
Bernabo, 2007	B	Spermatozoa	EV	Reproductive	50 Hz	1 mT	4 h	P	Two	Dye-Fluo3	Ic	nM	B	No
Bernabo, 2007	C	Spermatozoa	EV	Reproductive	50 Hz	1 mT	1 h	P	Two	45Ca	Ic	CMP	B	No
Bernabo, 2007	D	Spermatozoa	EV	Reproductive	50 Hz	1 mT	2 h	P	Two	45Ca	Ic	CMP	B	No
Bernabo, 2007	E	Spermatozoa	EV	Reproductive	50 Hz	1 mT	4 h	P	Two	45Ca	Ic	CMP	B	No

Morabito, 2010a	A	PC-12, Undifferentiated	CL	Neuro-endocrine	50 Hz	0.1 mT	30 min	D	Two	Dye-Fluo4	Co	% of total	S	No
Morabito, 2010a	B	PC-12, Differentiated	CL	Neuro-endocrine	50 Hz	1.0 mT	30 min	D	Two	Dye-Fluo4	Co	% of total	S	No
Morabito, 2010a	C	PC-12, Undifferentiated	CL	Neuro-endocrine	50 Hz	0.1 mT	30 min	D	Two	Dye-Fluo4	Co	% of total	S	No
Morabito, 2010a	D	PC-12, Differentiated	CL	Neuro-endocrine	50 Hz	1.0 mT	30 min	D	Two	Dye-Fluo4	Co	% of total	S	No
Morabito, 2010a	E	PC-12, Undifferentiated	CL	Neuro-endocrine	50 Hz	0.1 mT	7 d	P	Two	Dye-Fluo4	Ic	nM	S	No
Morabito, 2010a	F	PC-12, Differentiated	CL	Neuro-endocrine	50 Hz	1.0 mT	7 d	P	Two	Dye-Fluo4	Ic	nM	S	No
Morabito, 2010a	G	PC-12, Undifferentiated	CL	Neuro-endocrine	50 Hz	0.1 mT	7 d	P	Two	Dye-Fluo4	Ic	nM	S	No
Morabito, 2010a	H	PC-12, Differentiated	CL	Neuro-endocrine	50 Hz	1.0 mT	7 d	P	Two	Dye-Fluo4	Ic	nM	S	No
Garcia-Sancho, 1994*	-	Human Red blood cells	EV	Immune	29 Hz IRF	25, 100, 1000 μT	60 min	D	Two	45Ca	Ic	CMP	B	No
Garcia-Sancho, 1994*	-	Rat Thymocytes	EV	Immune	29 Hz IRF	25, 100, 1000 μT	60 min	D	Two	45Ca	Ic	CMP	B	No
Garcia-Sancho, 1994*	-	Ehrlich ascites tumor cells	EV	Epithelial	29 Hz IRF	25, 100, 1000 μT	60 min	D	Two	45Ca	Ic	CMP	B	No
Garcia-Sancho, 1994*	-	Human leukemia U937	CL	Immune	29 Hz IRF	25, 100, 1000 μT	60 min	D	Two	45Ca	Ic	CMP	B	No
Garcia-Sancho, 1994*	-	HL-60	CL	Immune	29 Hz IRF	25, 100, 1000 μT	60 min	D	Two	45Ca	Ic	CMP	B	No
Garcia-Sancho, 1994*	-	Rat spleen Lymphocytes	EV	Immune	11.6, 13.6, 15.6 Hz IRF	20 μT	60 min	D	Two	45Ca	Ic	CMP	B	No
Garcia-Sancho, 1994*	-	Rat Thymocytes	EV	Immune	12 – 20 Hz	20 μT	60 min	D	Two	45Ca	Ic	CMP	B	No
Lee, 2002*	-	CHO	CL	Ovary	55 – 60 Hz	1 μT	80 d	P	Two	Dye-Indo1	Ic	nM	B	No
Liburdy, 1993	A	Rat Thymic lymphocytes	EV	Immune	60 Hz	22 mT	60 min	D	Two	45Ca	Ic	CMP	B	Yes, ConA
Lisi, 2006	A	AtT20 D16V cells	CL	Neuro-endocrine	50 Hz	2 mT	6 min	D	Two	Dye-Indo1	Ic	nM	S	No
Kim, 2013	A	BM-MSCs	EV	Immune/stem cell	50 Hz	1 mT	12 d	P	Two	Dye-Fluo4	Ic	RFU	B	No

Piacentini, 2007	A	Neural stem/ progenitor cells	EV	Neural/stem cell	50 Hz	1 mT	3 d	P	Two	Dye-Fluo3	Co	Frequency	S	No
Piacentini, 2007	B	Neural stem/ progenitor cells	EV	Neural/stem cell	50 Hz	1 mT	3 d	P	Two	Dye-Fluo3	Ic	F1/F0	S	No
Piacentini, 2007	C	Neural stem/ progenitor cells	EV	Neural/stem cell	50 Hz	1 mT	6 d	P	Two	Dye-Fluo3	Co	% of total	S	No
Piacentini, 2007	D	Neural stem/ progenitor cells	EV	Neural/ stem cell	50 Hz	1 mT	6 d	P	Two	Dye-Fluo3	Ic	F1/F0	S	No
Loschinger, 1999	A	Human skin fibroblast	EV	Epithelial	20 Hz	8 mT	2560 sec	D	Two	Dye-Fluo3	Co	Events	S	No
Loschinger, 1999	B	Human skin fibroblast	EV	Epithelial	20 Hz	8 mT	2560 sec	D	Two	Dye-Fluo3	Co	Events	S	No
Loschinger, 1999	C	Human skin fibroblast	EV	Epithelial	20 Hz	8 mT	2560 sec	D	Two	Dye-Fluo3	Co	Events	S	No
Yamaguchi, 2002	A	MC3T3-E1	CL	Bone	120 Hz	0.6 mT	60 min	P	Two	Dye-Fura2	Ic	nM	S	No
Yamaguchi, 2002	B	MC3T3-E1	CL	Bone	30 Hz	0.6 mT	60 min	P	Two	Dye-Fura2	Ic	nM	S	No
Yamaguchi, 2002	C	MC3T3-E1	CL	Bone	30 Hz	1.0 mT	60 min	P	Two	Dye-Fura2	Ic	nM	S	No
Hwang, 2011*	-	RBL 2H3	CL	Immune	60 Hz	0.1 mT 1.0 mT	4 h 6 h	P	Two	Dye-Fura2	Ic	RFU	B	Yes, Melittin Ionomycin
Craviso, 2002	A	Chromaffin cells	EV	Neuro-endocrine	60 Hz	1.0 mT	10 min	D	Two	Dye-Fluo4	Co	Events	S	No
Craviso, 2002	B	Chromaffin cells	EV	Neuro-endocrine	60 Hz	1.4 mT	10 min	D	Two	Dye-Fluo4	Co	Events	S	No
Craviso, 2002	C	Chromaffin cells	EV	Neuro-endocrine	60 Hz	2.0 mT	10 min	D	Two	Dye-Fluo4	Co	Events	S	No
Craviso, 2002	D	Chromaffin cells	EV	Neuro-endocrine	60 Hz	1.0 mT	10 min	D	Two	Dye-Fluo4	Co	Events	S	No
Craviso, 2002	E	Chromaffin cells	EV	Neuro-endocrine	60 Hz	1.0 mT	10 min	D	Two	Dye-Fluo4	Co	Events	S	No
Lindström, 1995*	-	Jurkat	CL	Immune	5 Hz 100 Hz	0.15 mT	15 min	D	Own	Dye-Fura2	Co	Events	S	No
Lyle, 1997c	A	Jurkat	CL	Immune	60 Hz IRF	0.15 mT	10 min	D	Two	Dye-Fluo4	Co	% of total	S	No
Lyle 1997	B	Jurkat	CL	Immune	60 Hz IRF	0.15 mT	20 min	D	Two	Dye-Fluo3	Co	% of total	S	Yes, αCD3
Lyle, 1997	C	Jurkat	CL	Immune	60 Hz IRF	0.15 mT	2 min	D	Two	Dye-Fluo3	Co	% of total	S	Yes, αCD3
Lyle, 1997	D	Jurkat	CL	Immune	60 Hz IRF	0.15 mT	4 min	D	Two	Dye-Fluo3	Co	% of total	S	Yes, αCD3
Nishimura, 1999*		Thymocytes	EV	Immune	50 Hz	0.10 mT 0.14 mT	30 min	D	Own	Dye-Indo1	Ic	RFU	S	No

Coulton, 1993	A	T-cells	EV	Immune	16 Hz	47 μT	30 min	D	Two	Dye-Quin2	Ic	% increase over control	B	No
Coulton, 1993	B	T-cells	EV	Immune	16 Hz	44.4 μT	21 min	D	Two	Dye-Quin2	Ic	% increase over control	B	No
Coulton, 1993	C	T-cells	EV	Immune	16 Hz IRF	41.8 μT	21 min	D	Two	Dye-Quin2	Ic	% increase over control	B	No
Coulton, 1993	D	T-cells	EV	Immune	16 Hz	39.2 μT	15 min	D	Two	Dye-Quin2	Ic	% increase over control	B	No
Coulton, 1993	E	T-cells	EV	Immune	16 Hz	36.6 μT	32 min	D	Two	Dye-Quin2	Ic	% increase over control	B	No
Coulton, 1993	F	T-cells	EV	Immune	50 Hz	127.8 μT	3 min	D	Two	Dye-Quin2	Ic			
Coulton, 1993	G	T-cells	EV	Immune	50 Hz	119.7 μT	3 min	D	Two	Dye-Quin2	Ic	% increase over control	B	No
Coulton, 1993	H	T-cells	EV	Immune	50 Hz IRF	111.5 μT	45 min	D	Two	Dye-Quin2	Ic	% increase over control	B	No
Coulton, 1993	I	T-cells	EV	Immune	50 Hz	103.3 μT	45 min	D	Two	Dye-Quin2	Ic	% increase over control	B	No
Coulton, 1993	J	T-cells	EV	Immune	50 Hz	95.2 μT	45 min	D	Two	Dye-Quin2	Ic	% increase over control	B	No
Morabito, 2010b	A	C2C12, Myoblast	CL	Muscle	50 Hz	0.1 mT	27 min	D	Two	Dye-Fluo4	Co	% of total	S	No
Morabito, 2010b	B	C2C12, Myoblast	CL	Muscle	50 Hz	1 mT	27 min	D	Two	Dye-Fluo4	Co	% of total	S	No
Morabito, 2010b	C	C2C12, Myotube	CL	Muscle	50 Hz	0.1 mT	27 min	D	Two	Dye-Fluo4	Co	% of total	S	No
Morabito, 2010b	D	C2C12, Myotube	CL	Muscle	50 Hz	1 mT	27 min	D	Two	Dye-Fluo4	Co	% of total	S	No
Pilger, 2004	A	Fibroblast	EV	Epithelial	50 Hz	1 mT	7 h	P	Two	Dye-Fura2	Ic	nM	S	No
Pilger, 2004	B	Fibroblast	EV	Epithelial	50 Hz	1 mT	9 h	P	Two	Dye-Fura2	Ic	nM	S	No
Pilger, 2004	C	Fibroblast	EV	Epithelial	50 Hz	1 mT	11 h	P	Two	Dye-Fura2	Ic	nM	S	No
Pilger, 2004	D	Fibroblast	EV	Epithelial	50 Hz	1 mT	15 h	P	Two	Dye-Fura2	Ic	nM	S	No
Pilger, 2004	E	Fibroblast	EV	Epithelial	50 Hz	1 mT	17 h	P	Two	Dye-Fura2	Ic	nM	S	No
Walleczek, 1990	A	Thymocytes	EV	Immune	60 Hz	22 mT	60 min	D	Two	45Ca	Ic	CMP	B	Yes, ConA

Walleczek, 1990	B	Thymocytes	EV	Immune	60 Hz	22 mT	60 min	D	Two	45Ca	Ic	CMP	B	No
Grande, 1991[a]	-	Chondrocytes	EV	Bone	14.3 Hz IRF	40 µT	0.5-24 h	D	Two	45Ca	Ic	CMP	B	No
Galvanovskis, 1996	A	Jurkat E6.1	CL	Immune	50 Hz	100 µT	6 min	D	Own	Dye-Indo1	Co	Frequency	S	No
McCreary, 2006[c]	A	Jurkat E6.1 (TIB-152) G0/G1	CL	Immune	60 Hz	100 µT	6 min	D	Two	Dye-Indo1	Ic	F1/F0	B	No
McCreary, 2006	B	Jurkat E6.1 (TIB-152) G0/G1	CL	Immune	60 Hz	100 µT	6 min	D	Two	Dye-Indo1	Ic	F1/F0	B	Yes, αCD3
McCreary, 2006 [c]	C	Jurkat E6.1 (TIB-152) S	CL	Immune	60 Hz	100 µT	6 min	D	Two	Dye-Indo1	Ic	F1/F0	B	No
McCreary, 2006	D	Jurkat E6.1 (TIB-152) S	CL	Immune	60 Hz	100 µT	6 min	D	Two	Dye-Indo1	Ic	F1/F0	B	Yes, αCD3
McCreary, 2006[c]	E	Jurkat E6.1 (TIB-152) G2-M	CL	Immune	60 Hz	100 µT	6 min	D	Two	Dye-Indo1	Ic	F1/F0	B	No
McCreary, 2006	F	Jurkat E6.1 (TIB-152) G2-M	CL	Immune	60 Hz	100 µT	6 min	D	Two	Dye-Indo1	Ic	F1/F0	B	Yes, αCD3
McCreary,2006[c]	G	Jurkat E6.1 (TIB-152) G2-M	CL	Immune	60 Hz	100 µT	6 min	D	Two	Dye-Indo1	Ic	F1/F0	B	No
McCreary,2006	H	Jurkat E6.1 (TIB-152) G2-M	CL	Immune	60 Hz	100 µT	6 min	D	Two	Dye-Indo1	Ic	F1/F0	B	Yes, αCD3
McCreary,2002	A	Jurkat E6.1 (TIB-152) G2-M	CL	Immune	60 Hz	100 µT	7,5 min	D	Two	Dye-Indo1	Ic	Normalised	B	No
Luo, 2014[bc]	A	Entorhinal Cortex Neurons	EV	Neural	50 Hz	1 mT	5 /15 min 24 h	P	Two	Dye-Fluo4	Ic	RFU	S	No
Luo, 2014 [bc]	B	Entorhinal Cortex Neurons	EV	Neural	50 Hz	3 mT	5 /15 min 24 h	P	Two	Dye-Fluo4	Ic	RFU	S	No
Luo, 2014 [b]	C	Entorhinal Cortex Neurons	EV	Neural	50 Hz	1 mT	5 /15 min 24 h	P	Two	Dye-Fluo4	Ic	RFU	S	Yes, K^+
Luo, 2014 [b]	D	Entorhinal Cortex Neurons	EV	Neural	50 Hz	3 mT	5 /15 min 24 h	P	Two	Dye-Fluo4	Ic	RFU	S	Yes, K^+
de Groot, 2014	A	PC12 cells (CRL-1721)	CL	Neuro-endocrine	50 Hz	1 µT	30 min	D	Two	Dye-Fura2	Ic	RFU	S	No
de Groot, 2014	B	PC12 cells (CRL-1721)	CL	Neuro-endocrine	50 Hz	10 µT	30 min	D	Two	Dye-Fura2	Ic	RFU	S	No
de Groot, 2014	C	PC12 cells (CRL-1721)	CL	Neuro-endocrine	50 Hz	100 µT	30 min	D	Two	Dye-Fura2	Ic	RFU	S	No
de Groot, 2014	D	PC12 cells (CRL-1721)	CL	Neuro-endocrine	50 Hz	1000 µT	30 min	D	Two	Dye-Fura2	Ic	RFU	S	No
de Groot, 2014	E	PC12 cells (CRL-1721)	CL	Neuro-endocrine	50 Hz	1 µT	48 h	P	Two	Dye-Fura2	Ic	RFU	S	No
de Groot, 2014	F	PC12 cells (CRL-1721)	CL	Neuro-endocrine	50 Hz	10 µT	48 h	P	Two	Dye-Fura2	Ic	RFU	S	No
de Groot, 2014	G	PC12 cells (CRL-1721)	CL	Neuro-endocrine	50 Hz	100 µT	48 h	P	Two	Dye-Fura2	Ic	RFU	S	No

de Groot, 2014	H	PC12 cells (CRL-1721)	CL	Neuro-endocrine	50 Hz	1000 μT	48 h	P	Two	Dye-Fura2	Ic	RFU	S	No
Liu, 2014*	A	Cerebellar granule cells	EV	Neural	50 Hz	1 mT	60 min	P	Two	Dye-Fura2	Ic	Normalised	S	Yes, K^+
Liu, 2014	B	Cerebellar granule cells	EV	Neural	50 Hz	1 mT	60 min	P	Two	Dye-Fura2	Ic	Normalised	S	Yes, Melatonin and K^+
Wu, 2014	A	Amnionic epithelial cells	CL	Epithelial	50 Hz	0.4 mT	30 min	P	Two	Dye-Fluo3	Ic	RFU	B	No
Wei, 2014	A	Cardiomyocytes	EV	Muscle	15 Hz	2 mT	3 min	D	Own	Dye-Fura2	Ic	RFU	B	No
Wei, 2014	B	Cardiomyocytes	EV	Muscle	50 Hz	2 mT	3 min	D	Own	Dye-Fura2	Ic	RFU	B	No
Wei, 2014	C	Cardiomyocytes	EV	Muscle	75 Hz	2 mT	3 min	D	Own	Dye-Fura2	Ic	RFU	B	No
Wei, 2014	D	Cardiomyocytes	EV	Muscle	100 Hz	2 mT	3 min	D	Own	Dye-Fura2	Ic	RFU	B	No
Wei, 2014 [c]	E	Cardiomyocytes	EV	Muscle	15 Hz	2 mT	3 min	D	Own	Dye-Fura2	Co	Amplitude	B	No
Wei, 2014 [c]	F	Cardiomyocytes	EV	Muscle	50 Hz	2 mT	3 min	D	Own	Dye-Fura2	Co	Amplitude	B	No
Wei, 2014 [c]	G	Cardiomyocytes	EV	Muscle	75 Hz	2 mT	3 min	D	Own	Dye-Fura2	Co	Amplitude	B	No
Wei, 2014 [c]	H	Cardiomyocytes	EV	Muscle	100 Hz	2 mT	3 min	D	Own	Dye-Fura2	Co	Amplitude	B	No
Wei, 2014	I	Cardiomyocytes	EV	Muscle	15 Hz	2 mT	3 min	D	Own	Dye-Fura2	Co	Frequency	B	No
Wei, 2014	J	Cardiomyocytes	EV	Muscle	50 Hz	2 mT	3 min	D	Own	Dye-Fura2	Co	Frequency	B	No
Wei, 2014	K	Cardiomyocytes	EV	Muscle	75 Hz	2 mT	3 min	D	Own	Dye-Fura2	Co	Frequency	B	No
Wei, 2014	L	Cardiomyocytes	EV	Muscle	100 Hz	2 mT	3 min	D	Own	Dye-Fura2	Co	Frequency	B	No
Lyle, 1991	A	CTLL-1	CL	Immune	13.6 Hz IRF	20 μT	30 min	D	Two	45Ca	Ic	CMP	B	No
Lyle, 1991	B	Spleen lymphocytes	EV	Immune	13.6 Hz IRF	20 μT	30 min	D	Two	45Ca	Ic	CMP	B	No
Lyle, 1991	C	EL4	CL	Immune	13.6 Hz IRF	20 μT	30 min	D	Two	45Ca	Ic	CMP	B	No
Lyle, 1991	D	Spleen lymphocytes	EV	Immune	60 Hz	20 μT	30 min	D	Two	45Ca	Ic	CMP	B	No

* Data incomplete, excluded from meta-analysis. [a] Categorised MF duration: min. [b] Categorised MF duration: days. [c] Not independent measurements, included only in subgroup analyses and excluded from overall meta-analysis.

Table S2. Assessment of bias risk per included study

Risk description	Mattsson 2001 [44]	Wey 2000 [45]	Conti 1985a [12]	Lindström 1998 [46]	Tonini 2001 [32]	Liburdy 1992 [24]	Craviso 2003 [47]	Fitzsimmons 1994 [48]
Exposure with dummy/sham system? [a]	Yes	Yes	No	Yes	Yes	Yes	Yes	No
Duration of exposure described?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Frequency described?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	yes
Magnetic flux density described?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Cell type described?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Temperature controlled during exposure? [b]	Partly	Partly	Partly	Partly	Yes	Yes	Partly	Yes
Reported temperature value	37	37	37	37	37±0.6	37±0.05	31--	37±0.1
Background MF reported?	Partly	Yes	No	Partly	No	Yes	Yes	Yes
Background magnetic fields (50/60 Hz)	-	0.14 μT	-	-	-	0.5 μT	1 μT	15±1.6 μT
Static background magnetic fields	60 μT	0.7 μT	-	64 – 60 μT	-	20.5 μT	75 μT	20 μT
Blinding of exposure applied?	unclear	Yes	unclear	unclear	unclear	unclear	unclear	unclear
Randomise data measurements?	unclear	Yes	unclear	unclear	unclear	unclear	unclear	unclear
Randomisation of exposure applied?	unclear	Yes	unclear	unclear	unclear	unclear	unclear	unclear
Methods used the same? [d]	Partly	Yes	Yes	Partly	Yes	Yes	Yes	Yes
Cell vitality scored/measured?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	unclear
Industry sponsoring involved? [e]	No	No	No	No	No	No	No	Yes

[a] Dummy/sham is with the use of an unenergised coil or cancelling sham exposure

[b] Yes = If the temperature is defined with a SD or SE range. Partly = only the mean temperature value. No = no value

[c] Yes = Background fields reported. Partly = Only 50/60 Hz or static field value reported. No = Background MFs not reported

[d] Were the methods used to measure calcium homeostasis the same for the exposure and control sample. Partly = When an independent measurement was performed, when the sham exposure period was measured before exposure, in the same cell or batch of cells. Yes = When control/sham and exposure were two separate groups

[e] Was industry sponsoring involved: Yes = increased risk of bias.

	Fixler 2012 [49]	Sakurai 2005 [30]	Gaetani 2009 [50]	Oh 2001 [39]	Conti 1985b [40]	Bernabo 2007 [51]	Morabito 2010a [52]	Garcia-Sancho 1994 [33]
Exposure with dummy system?	Yes	Yes	Yes	unclear	unclear	unclear	Yes	No
Duration of exposure clear?	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Frequency clearly described?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Magnetic flux density clearly described?	Yes	Yes	Yes	Yes	Partly	Yes	Yes	Yes
Cell type mentioned?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Temperature controlled during exposure?	Partly	Partly	Yes	Partly	Partly	Partly	Partly	Partly
Temperature	20-39	37	37±0.1	37	37	38.5	37	37
Background MF reported?	Partly	No	No	Partly	No	Partly	Partly	Yes
Background magnetic fields (50/60 Hz)	-	-	-	-	-	-	0.7 μT	0.2 μT
Static background magnetic value	40-50 μT	-	-	Negligible	-	38 μT	-	41 μT
Blinding of exposure?	unclear	unclear	Yes	unclear	unclear	unclear	unclear	unclear
Randomise data measurements?	unclear	unclear	unclear	unclear	unclear	unclear	unclear	unclear
Randomisation of exposure?	unclear	unclear	unclear	unclear	unclear	unclear	unclear	unclear
Methods used the same in both groups?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Cell vitality scored/measured?	unclear	Yes	Yes	Yes	Yes	Yes	Yes	unclear
Industry Sponsoring involved?	No	No	No	No	No	No	No	No

	Lee 2002 [34]	Liburdy 1993 [53]	Lisi 2006 [28]	Kim 2013 [54]	Piacentini 2008 [55]	Loschinger 1999 [56]	Yamaguchi 2002 [57]	Hwang 2011 [35]	Craviso 2002 [58]
Exposure with dummy system?	unclear	Yes	Yes	unclear	No	Yes	Yes	unclear	Yes
Duration of exposure clear?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Frequency clearly described?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Magnetic flux density clearly described?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Cell type mentioned?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Temperature controlled during exposure?	Partly	Yes	Yes	Partly	Yes	Yes	Yes	Yes	Yes
Temperature	37	37.0±0.05	37±0.5	37	37±0.4	37±0.2	37±0.6	37±0.3	32±0.1
Background MF reported?	No	Yes	No	No	No	Yes	No	No	Yes
Background magnetic fields (50/60 Hz)	-	0.1 μT	-	-	-	0.25 μT	-	-	0.1 μT
Static background magnetic value	-	20.5 μT	-	-	-	41 μT	-	-	75 μT
Blinding of exposure?	unclear	unclear	Yes	unclear	unclear	unclear	unclear	unclear	unclear
Randomise data measurements?	unclear	unclear	unclear	unclear	unclear	unclear	unclear	unclear	Yes
Randomisation of exposure?	unclear	unclear	unclear	unclear	unclear	unclear	unclear	unclear	unclear
Methods used the same in both groups?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Cell vitality scored/measured?	Yes	Yes	Yes	Yes	Yes	Yes	unclear	Yes	Yes
Industry Sponsoring involved?	No	No	Yes	No	No	No	Yes	Yes	No

	Lindström 1995 [36]	Lyle 1997 [59]	Nishimura 1999 [37]	Coulton 1993 [60]	Morabito 2010b [61]	Pilger 2004 [29]	Walleczek 1990 [27]	Grande 1991 [38]	Galvanovskis 1996 [26]
Exposure with dummy system?	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes
Duration of exposure clear?	Partly	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Frequency clearly described?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Magnetic flux density clearly described?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Cell type mentioned?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Temperature controlled during exposure?	Partly	Partly	Yes	Partly	No	Yes	Yes	No	No
Temperature	37	37	37±0.1 µT	36.8	RT	37±0.3	37±0.1	-	RT
Background MF reported?	Yes	Partly	Yes	Yes	No	Partly	Yes	No	Yes
Background magnetic fields (50/60 Hz)	0.2 µT	-	0.08 µT	50-240 nT	-	µ metal	1 µT	-	1.05-0.62 µT
Static background magnetic value	64 µT	78.2 µT	42.3 µT	5 - 8.2 µT	-	µ metal	44 µT	-	22.2 µT
Blinding of exposure?	unclear	unclear	unclear	unclear	unclear	Yes	unclear	unclear	unclear
Randomise data measurements?	unclear	unclear	unclear	unclear	unclear	unclear	unclear	unclear	unclear
Randomisation of exposure?	unclear	unclear	unclear	unclear	unclear	Yes	unclear	unclear	unclear
Methods used the same in both groups?	Yes	Yes	Partly	Yes	Yes	Yes	Yes	Yes	Partly
Cell vitality scored/measured?	Yes	Yes	Yes	Yes	Yes	unclear	Yes	Yes	Yes
Industry Sponsoring involved?	No	No	Yes	No	No	No	No	Yes	No

	McCreary 2006 [10]	McCreary 2002 [62]	Luo 2014 [41]	de Groot 2014 [63]	Liu 2014 [31]	Wu 2014 [65]	Wei 2014 [64]	Lyle 1991 [66]
Exposure with dummy system?	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Duration of exposure clear?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Frequency clearly described?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Magnetic flux density clearly described?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Cell type mentioned?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Temperature controlled during exposure?	Partly	Yes	Yes	No	Yes	Partly	Yes	Partly
Temperature	37	37±0.1	37.0 – 37.5	-	37.0 ± 0.4	37	37±0.1	37
Background MF reported?	Partly	Partly	No	Partly	No	No	No	Partly
Background magnetic fields (50/60 Hz)	-	-	-	0.2 µT	-	-	-	-
Static background magnetic value	0±0.5 µT	78.1±0.3 µT	-	-	-	-	-	16.5±0.5 µT
Blinding of exposure?	No	unclear	unclear	unclear	unclear	Yes	unclear	unclear
Randomise data measurements?	Yes	unclear	unclear	unclear	unclear	unclear	unclear	unclear
Randomisation of exposure?	unclear	unclear	unclear	unclear	unclear	unclear	Yes	unclear
Methods used the same in both groups?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Cell vitality scored/measured?	Yes	unclear	unclear	unclear	unclear	Yes	unclear	unclear
Industry Sponsoring involved?	No	No	No	No	No	No	No	No

Table S2

Table S3. Applied magnetic field and induced electric field

Excel file available upon request

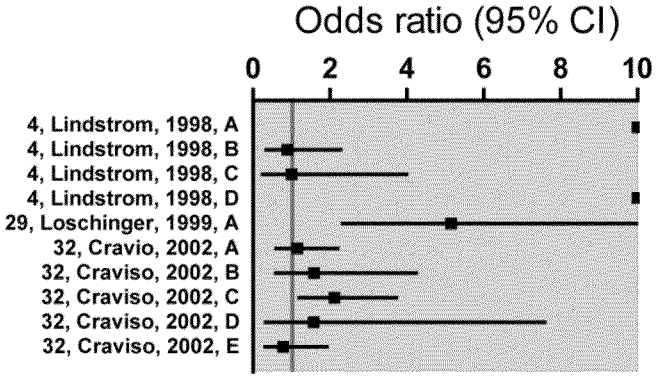

Figure S1. Occurrence of calcium oscillations during MF or control treatment. Number of events expressed as odds ratio and 95% confidence interval.

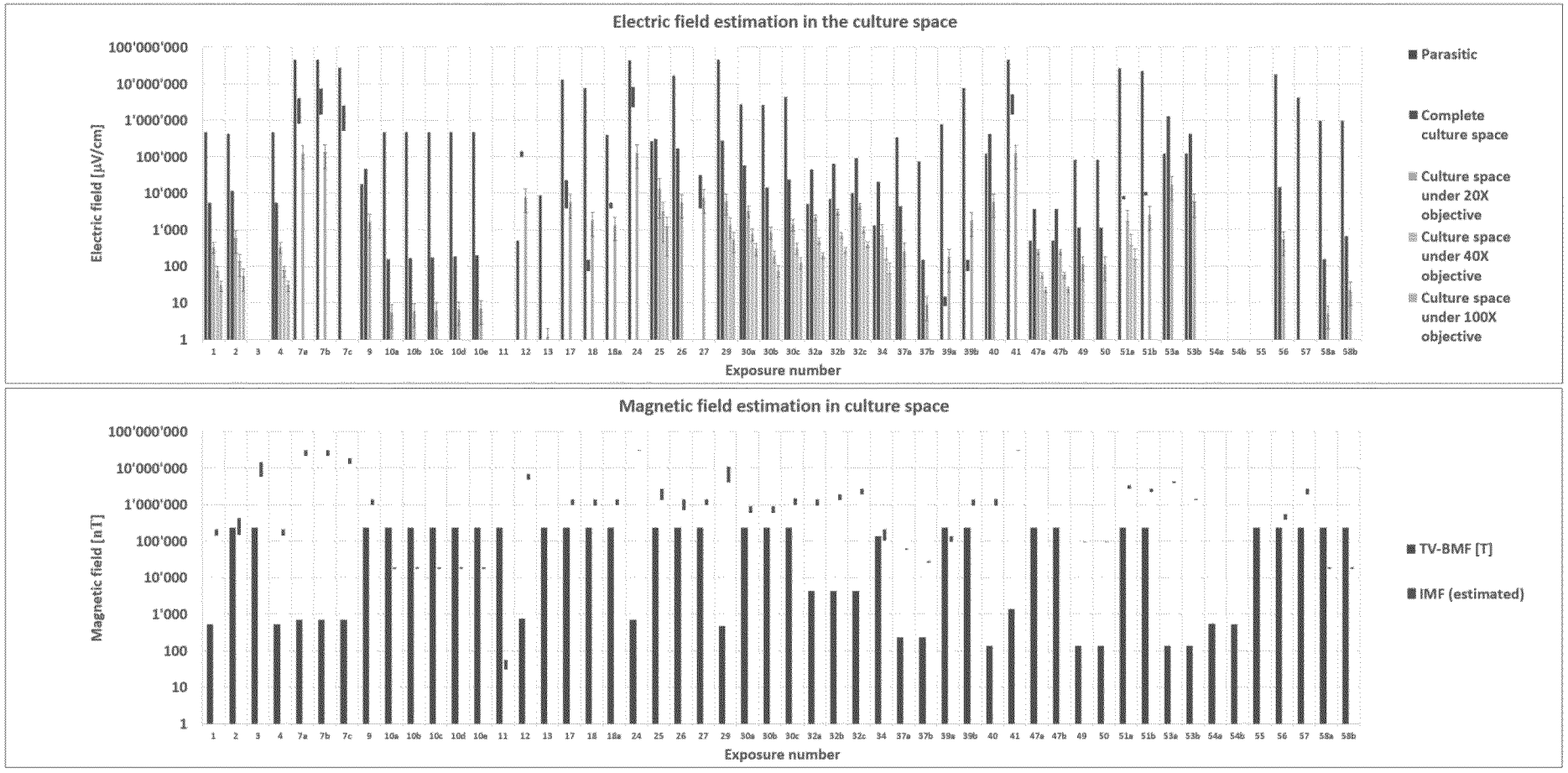

Figure S2: Estimation of electric and magnetic field exposure parameters . Bars denote the range over which the actual exposure values can be found for all portions of the culture space considered based on the reported exposure system and culture container characteristics. A complete description of the assumptions, estimations, and calculations performed is found in Supplementary Note 1 . Note how artefacts have the potential to dominate the desired exposure parameters for many cases. IMF: Imposed magnetic field; TV-BMF: Artificially generated time-varying background magnetic field (50 and 60 Hz only).

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